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# MYCOTAXON

AN INTERNATIONAL JOURNAL FOR RESEARCH ON
TAXONOMY & NOMENCLATURE OF FUNGI, INCLUDING LICHENS

Volume LXXVI October-December 2000

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# ADDITIONAL NOTES ON SARCOSCYPHA IN CHINA*

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**Abstract**: A new species and a new Chinese record of *Sarcoscypha* (Pezizales) are reported from China. The known distribution of some species of the genus in China and in the world is briefly discussed.

**Key words**: *Sarcoscypha mesocyatha*; *Sarcoscypha shennongjiana*; distribution.

*Sarcoscypha* (Fr.: Fr.) Boud., containing the well-known beautiful scarlet elf cup, is distributed widely in different regions of China. Eight species of the genus have been reported (Teng, 1947, 1963; Zang, 1983; Zhuang, 1991, 1993; Wang & Zhuang, 1996; Harrington, 1997). A recent collection from the Daweishan Natural Reserve, Pingbian County in tropical Yunnan and the other from Guizhou were identified as *Sarcoscypha mesocyatha* F. A. Harr. which was known previously only from type locality, Hawaii, USA (Harrington, 1997). Our collections extend the distribution of the species to tropical and subtropical China. Several other collections from Shennongjia, Hubei Province, a subtropical region, were re-examined. They turn out to be a new species.

**Sarcoscypha mesocyatha** F. A. Harr., Harvard Pap. Bot. 10: 62, 1997. 平盘肉杯菌 (Figs. 1, 3a, b)

Diagnostic features: Apothecia solitary to gregarious, surface flat to convex, often undulate, sessile to subsessile, sometimes eccentrically attached, 10-27 mm in diam; hymenium Scarlet Red when fresh and Scarlet to Perch Red when dry (Ridgway), receptacle whitish with a red tint when fresh and paler when dry, surface wrinkled when dry. Ectal excipulum of *textura prismatica* to *textura porrecta*, ca 55 μm thick; cells 20-30 x 5.5-10 μm. Medullary excipulum of *textura intricata*, 460 μm thick at flanks between margin and apothecial base, thicker near the base, hyphae hyaline, 5-9 μm wide. Subhymenium 40-60 μm thick, cell contents with red pigmentation. Hymenium 255-270 μm thick. Asci 8-spored, mostly 12-12.7 μm wide. Ascospores ellipsoid to cylindric-ellipsoid, most of them with deep depression at both ends, flattened laterally when viewed from the end, 19-27.5 x 7.5-11.5 μm; biguttulate, guttules 5.5-8 μm in diam. Paraphyses filiform, with red pigmentation, ca 2 μm wide.

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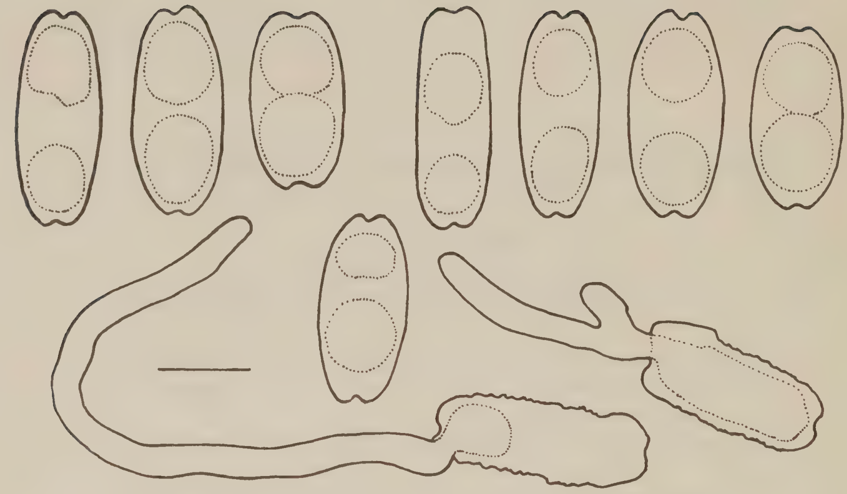

Fig. 1. *Sarcoscypha mesocyatha*: Ascospores and spore germination, left three on top from holotype and the rest from HMAS 78137; scale = 10 μm.

Chinese material examined: on rotten wood, Daweishan, Pingbian County, Yunnan, alt. 1600 m, 4 XI 1999, W. Y. Zhuang & Z. H. Yu 3261, HMAS 78137; on rotten twig, Guizhou, 11 X 1993, S. C. He 1559, HMAS 71947; on twig, Qingchengshan, Sichuan, 5 X 1999, Z. L. Yang 2780, HKAS 34246, HMAS 76095.

Other specimens examined: on *Acacia koa*, Kokee State Park, Kauai, Hawaii, USA, 7 I 1995, D. Hemmes, CUP 63490 (holotype); on dead twigs, mostly under *Acacia koa* and *Caracas* sp., Kokee State Park, Kauai, Hawaii, USA, 7 I 1994, G. J. Wong 1242, CUP 62699.

Notes: The holotype specimen of *Sarcoscypha mesocyatha* (CUP 63490) and another collection (CUP 62699) from Hawaii, USA were examined and compared with the Chinese collections. Significant distinctions were not found. Ascospore guttules of all collections are large. Presence of deep depression at both end of spores is one of the diagnostic features of the fungus. Color of hymenium seems slightly different both in fresh and in dry conditions between collections from two geographical regions, especially when dry (American collections: lemon, light orange, to persian orange, Flame Scarlet, or Mikado Orange; Chinese one: Scarlet to Perch Red). Germ tubes produced from one end of the spores upon germination in the Yunnan collection (Fig. 1).

Three types of spore surface morphology have been recognized in *Sarcoscypha* (Zhuang, 1993; Wang & Zhuang, 1996), *i.e.* cerebriform as shown in *S. cerebriformis* W. Y. Zhuang & Z. Wang, wrinkled with irregular deep grooves in *S. striatispora* W. Y. Zhuang, and smooth to slightly roughened in most species. SEM photographs reveal that ascospore surface of *S. mesocyatha* belongs to the smooth or slightly roughened type. The spore surface of the Chinese collection looks smoother than that of the American one (Fig. 3a, b). Spore shapes are usually indistinguishable among collections either under light microscope or in SEM study.

**Sarcoscypha shennongjiana** W. Y. Zhuang, **sp. nov.** 神农架肉杯菌 (Figs. 2, 3c, d)

Ab *Sarcoscypha coccinea* ascis parvulis, 240-292 x (9.5-)10-13.5 μm; ascosporis parvulis, truncatus, biguttulatis, 18-26.7(-30) x (8-)9-11.6(-12) μm differt.

Apothecia discoid to cupulate, 10-50 mm in diam, sessile to stipitate; stipes 1.5-10 mm long, centrally or eccentrically attached, with subiculum of 2.5-5 mm in diam at base when dry; hymenium orange red to red when fresh, Capucine Orange, Ochraceous-Buff, Ochraceous-Orange, Ochraceous-Salmon, Xanthine Orange, or Tawny when dry; receptacle surface whitish when fresh, Orange Buff, Orange-Cinnamon, Ochraceous-Orange, Xanthine Orange, Mars Yellow, between Capucine Buff and Capucine Orange, or Tawny (Ridgway, 1912) and strongly wrinkled when dry. Ectal excipulum of *textura porrecta* (to *textura prismatica* in very outer cell layer), 50-75 μm thick; cells 10-35 x 2-7 μm. Medullary excipulum of *textura intricata*, 150-410 μm thick at flanks near margin, thicker at lower flanks; hyphae hyaline, 2-6 μm wide. Subhymenium not clearly distinguished. Hymenium 240-310 μm. Asci suboperculate, 8-spored, 240-292 x (9.5-)10-13.5 μm. Ascospores ellipsoid with shallow depression at truncate ends, somewhat flattened laterally when viewed from the end, occasionally a protrusion appearing in the middle of depression either at one end or at both ends of spores, with two large guttules up to 7.5 μm in diam and many small ones, 18-26.7(-30) x (8-)9-11.6(-12) μm. Paraphyses subcylindrical, 2-3 μm wide.

Holotype: on twigs of a broadleaf tree, Motianling, Shennongjia, Hubei, alt.1700 m, 26 VIII 1984, J. X. Tian 188, HMAS 53675.

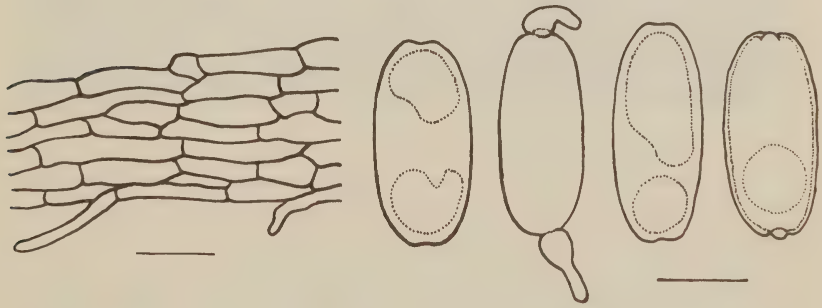

Fig. 2. *Sarcoscypha shennongjiana*: **a**. Ectal excipular structure, from holotype, scale = 20 μm; **b**. Ascospores and spore germination, left three from holotype and right one from HMAS 53676, scale = 10 μm.

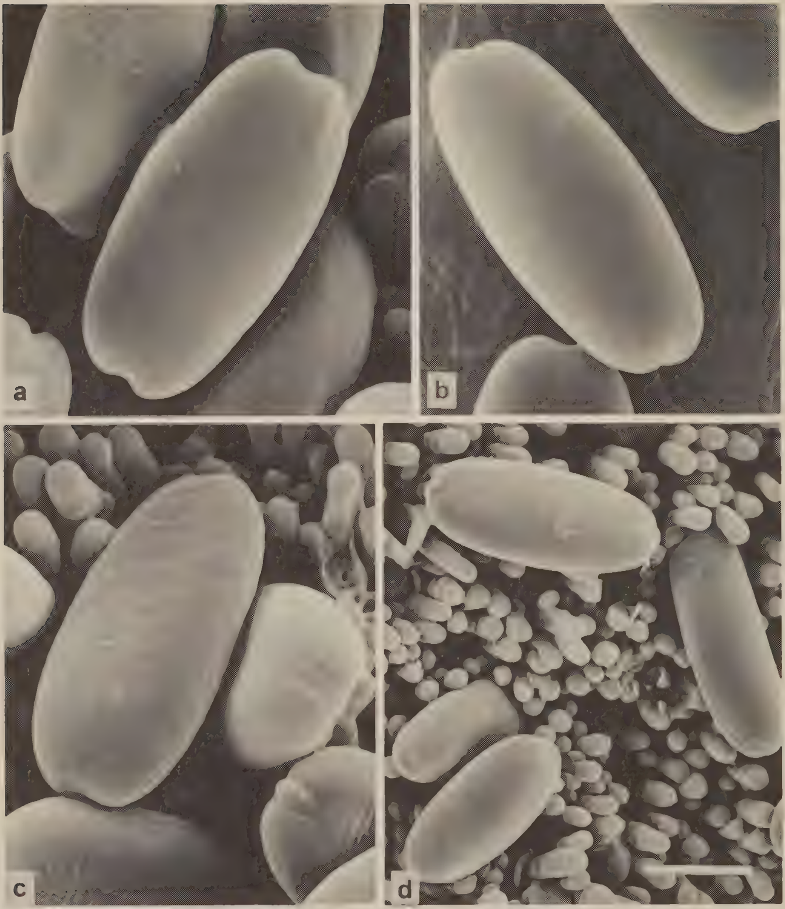

Fig. 3. SEM photographs of *Sarcoscypha* ascospores. **a**, **b**. *S. mesocyatha*. **a** from holotype, **b** from HMAS 78137. **c**, **d**. *S. shennongjiana*. **c** from holotype, **d** from HMAS 53676. Scale = 5 µm (**a**, **b**, **c**); 10 µm (**d**).

Paratypes: on twigs, Laojunshan, Shennongjia, Hubei, 11 VIII 1984, J. X. Tian 132-2, HMAS 53674; on twigs, Shennongjia, Hubei, 29 VIII 1984, J. X. Tian, HMAS 53676; on twigs, Shennongjia, Hubei, 29 VIII 1984, S. X. Sun & X. Q. Zhang 800, HMAS 70107.

Notes: The fungus was formerly recorded as *Sarcoscypha coccinea* (Scop.: Fr.) Lambotte complex (Zhuang, 1989) and *S.* near *coccinea* (Wang & Zhuang, 1996).

Detailed studies indicated that it is different from *S. coccinea* (Harrington, 1990) in apothecia with longer stipes, asci smaller, ascospores smaller, ellipsoid, with shallow depression at truncate ends and two large polar guttules. A protrusion positioned in the middle of spore depression has seldom been reported from *Sarcoscypha*. Although it occurred very rarely in most collections studied, about 5% of spores in HMAS 53676 show the characteristic, which was observed clearly both under the light microscope and in SEM study. SEM photographs reveal that ascospores of *S. shennongjiana* has smooth to very slightly roughened surface. The above features are sufficiently distinct to separate the Shennongjia fungus from the existing taxa of the genus.

## Known distribution of some species of *Sarcoscypha*

*Sarcoscypha* is wide-spread in the world (Le Gal, 1953; Rifai, 1968; Gamundí, 1971; Baral, 1984; Harrington, 1990, 1997; Zhuang, 1991, 1993, this paper; Wang & Zhuang, 1996). *S. coccinea* is cosmopolitan. Published information indicates that Asia has the highest population of the genus and is rich in species. Twelve taxa have been found in the continent (Table 1).

Table 1. Known distribution of some species of *Sarcoscypha* in the world.

| | |
|---|---|
| Asia | *S. cerebriformis*, *S. coccinea*, *S. hosoyae*, *S. humberiana*, *S. knixoniana*, *S. korfiana*, *S. mesocyatha*, *S. occidentalis*, *S. shennongjiana*, *S. sherriffii*, *S. striatispora*, *S. vassiljevae* |
| Europe | *S. austriaca*, *S. coccinea*, *S. emarginata*, *S. macaronesica* |
| Australia | *S. coccinea*, *S. mesocyatha* |
| Africa | *S. coccinea*, *S. serrata* |
| N. America | *S. austriaca*, *S. coccinea*, *S. dudleyi*, *S. occidentalis* |
| S. America | *S. coccinea* |

Table 2. Known distribution and time of fructification of *Sarcoscypha* in China.

| | |
|---|---|
| *S. cerebriformis* | Tibet, Yunnan (September, October) |
| *S. coccinea* | Guangxi, Hunan, Yunnan (September through April) |
| *S. humberiana* | Taiwan (March) |
| *S. korfiana* | Jilin (July, August) |
| *S. mesocyatha* | Guizhou, Sichuan, Yunnan (October, November) |
| *S. occidentalis* | Gansu, Jiangxi, Jilin, Shaanxi, Taiwan, Yunnan (July to Sept.) |
| *S. shennongjiana* | Hubei (August) |
| *S. sherriffii* | Tibet (March) |
| *S. striatispora* | Guizhou, Yunnan (August) |
| *S. vassiljevae* | Beijing, Heilongjiang, Jilin (August, September) |

Compared with the other Asian countries, China has the highest species diversity. Members of *Sarcoscypha* are distributed widely throughout China. Ten species have been found. *S. occidentalis* is the most common species (Table 2). Yunnan Province where plant species are the richest in China has the highest species diversity of *Sarcoscypha*; Jilin in the northeast ranks the next (Fig. 4). Further investigations will prove that species diversity of the genus in this country is even higher than what we currently know. Questions raised here are where the genus originated and whether geographic separation created species.

## ACKNOWLEDGEMENTS

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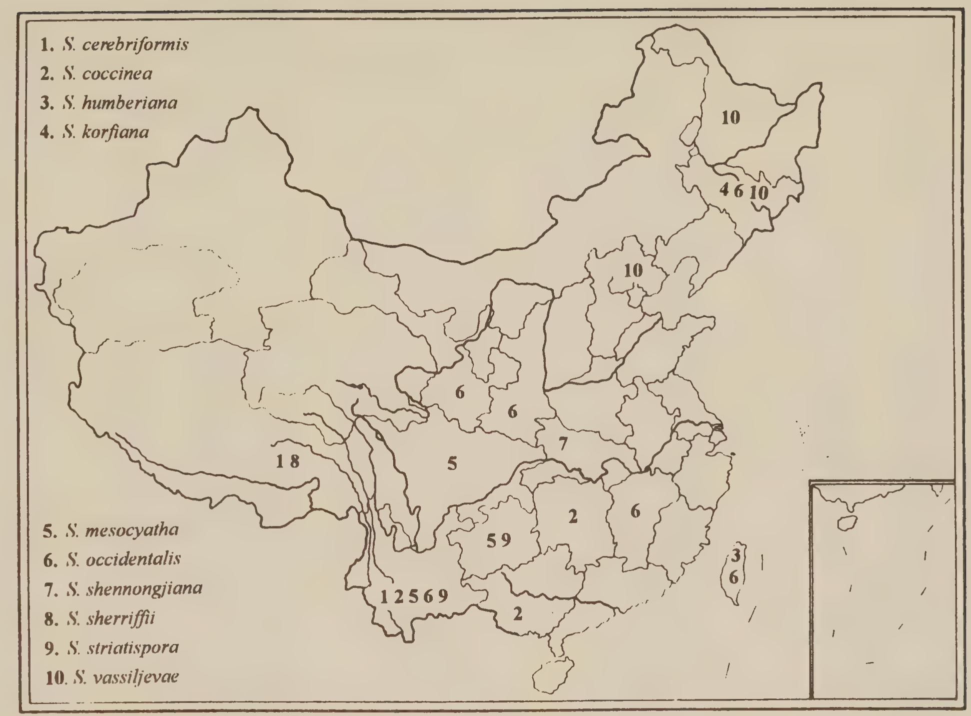

Fig. 4. Map of China showing distribution of *Sarcoscypha*.

管理情况
- 总则
- 实验室的研究方向及课题申请指南
- 课题研究经费分配原则
- 课题管理
- 设备、资料及成果管理
- 经费使用原则
- 分子系统学实验室使用规则

## 四、上次评估存在问题与改进，目前存在问题与改进建议

MYCOTAXON

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# MORPHOLOGICAL AND MOLECULAR DATA ON THE TAXONOMY OF GYMNOMYCES, MARTELLIA AND ZELLEROMYCES (RUSSULALES)

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**Abstract**.- This article reviews the current taxonomy of the family *Elasmomycetaceae*. After examining numerous Spanish specimens belonging to the genera *Gymnomyces, Martellia* and *Zelleromyces,* a series of suggestions are made to improve the delimitation among the three genera. Preliminary data based on molecular analysis of the rDNA gene, confirm the relationship between these genera and the epigeous Russulales.
Key words: *Elasmomycetaceae, Russulales,* taxonomy, ITS rDNA

**Résumé**.- Cet article est a tentative pour la clarification de la situation actuelle de la taxonomie dans la famille *Elasmomycetaceae*. Après l´examen de materiel espagnol des genres *Gymnomyces, Martellia* et *Zelleromyces,* nous proposons suggestions pour amender la délimitation entre tous les trois genres. Les premiers données basées sur l'analyse moléculaire de rDNA ont confirmé les relations entre ces genres et les Russulales epigés.
Mots clé: *Elasmomycetaceae, Russulales,* taxonomie, ITS rDNA

**Resumen**.- Este artículo intenta aclarar, en parte, la situación actual de la taxonomía de la familia *Elasmomycetaceae*. Despues del estudio de numerosas muestras de material español, de los géneros *Gymnomyces, Martellia* y *Zelleromyces,* se proponen sugerencias, dirigidas a mejorar la delimitación entre los tres géneros. Los primeros datos, basados en el análisis de las secuencias del gen rDNA, confirman las relaciones filogenéticas entre estos géneros y los Russulales epigeos.
Palabras clave: *Elasmomycetaceae, Russulales,* taxonomía, ITS rDNA

## INTRODUCTION

The taxonomy of fungi is a difficult task, and the gasteroid *Russulales* do not escape this problem. The order *Russulales* embraces two families: *Russulaceae* and *Elasmomycetaceae,* the basic differences being in the type of spores. The spores of *Russulaceae* are ballistosporic and asymmetric, while in *Elasmomycetaceae* the spores are statismosporic and symmetric. The latter family embraces four genera: *Elasmomyces, Gymnomyces, Martellia* and *Zelleromyces.* While *Elasmomyces* is easily separable by having basidioma stipitate and gleba exposed at maturity, the other three genera are sessile with gleba not exposed at maturity (PEGLER & YOUNG, 1979).

Differentiating among these three genera is almost impossible when working with dried specimens. Thus, the present paper proposes some ideas to improve the identification of genera *Gymnomyces, Martellia* and *Zelleromyces.*

The use of molecular techniques in fungi provides new information in taxa where, as in this group, the morphological identification is ambiguous (MARTÍN & ROCABRUNA, 1999; JOHANNESSON & MARTÍN, 1999). Thus, in the present work, through the sequence analysis of the internal transcribed spacer regions of rDNA (ITS1 and ITS2, including the 5.8S), we examine the boundaries between the three genera and the relationships with *Lactarius* and *Russula* species.

## MATERIAL AND METHODS

### *Material*

The material used here have all been collected in Spain, and previously described in other papers (CALONGE & PEGLER, 1998; MORENO-ARROYO *et al.,* 1999). New collections of *Russula* and *Lactarius* have been included in the molecular analysis (Table 1). All the specimens are preserved at the Madrid Botanic Garden herbarium (MA-Fungi).

**Table 1**. List of specimens used in the molecular analysis.

| Taxon name | MA-Fungi | GenBank AccNum. |
|---|---|---|
| *Gymnomyces ammophilus* Vidal & Calonge | 40132 | AF230890 |
| *Gymnomyces ammophilus* Vidal & Calonge | 40137 | AF230891 |
| *Martellia pila* (Pat.) Vidal | 30667 | AF230893 |
| *Martellia pila* (Pat.) Vidal | 30802 | AF230894 |
| *Russula foetens* Fr. | 42065 | AF230895 |
| *Russula fragilis* (Pers.: Fr.) Fr. | 42067 | AF230898 |
| *Russula integra* L. : Fr. | 42066 | AF230896 |
| *Russula mairei* Sing. | 42069 | AF230899 |
| *Russula postiana* Romagn. | 42068 | AF230897 |
| *Zelleromyces giennensis* Calonge, Moreno-Arroyo & Gómez | 38674 | AF230800 |
| *Zelleromyces hispanicus* Calonge & Pegler | 37497 | AF231911 |
| *Zelleromyces hispanicus* Calonge & Pegler | 37498 | AF231912 |
| *Zelleromyces hispanicus* Calonge & Pegler | 38311 | AF231913 |
| Outgroup | | |
| *Hygrophorus latitabundus* Britz. | 42071 | AF231915 |
| *Suillus variegatus* (Swartz: Fr) O. Kuntze | 42070 | AF231914 |

### *Molecular methods*

Total DNA was isolated with E.Z.N.A. Fungi DNA miniprep kit (Omega Biotech) as described in MARTÍN & GARCÍA-FIGUERES (1999). Amplifications were done with Ready-To-Go® PCR Beads (Amersham-Pharmacia Biotech) (WINKA et al, 1998) and the primer pair ITS1F/ ITS4 (WHITE et al.,1990). Amplification products were cleaned using E.Z.N.A. Clean kit (Omega Biotech) and both strands were sequenced separately using primers ITS1F and ITS4 with and

ABI Prism 377 Genetic Analyzer and the ABI Prism™ BigDye™ Terminator Cycle Sequencing Ready reaction Kit with AmpliTaq®DNA Polymerase (Perkin Elmer Applied Biosystem).

*Sequencing and phylogenetic analysis*

The software used for sequencing analysis is described in MARTÍN & GARCÍA-FIGUERES (1999). The new sequences have been logged in the EMBL database with the accession number indicated in Table 1. A sequence of *Lactarius deliciosus* was retrieved from the GenBank (U80999) and compared with the homologous sequence obtained in this study. Parsimony analysis was performed using the computer program PAUP 3.0s (Phylogenetic Program Using Parsimony) of SWOFFORD (1991). Isolates of *Suillus variegatus* and *Hygrophorus latitabundus* (Table 1) were used of outgroup. Branch robustness was estimated by boostrap analysis (FELSENTEIN, 1985) of 1000 heuristic replicates.

## RESULTS AND DISCUSSION

After the excellent work published by SINGER & SMITH (1960), where they established the basis to separate the genera *Gymnomyces, Martellia* and *Zelleromyces,* several other useful contributions have further clarified the taxonomy of these genera. PEGLER & YOUNG (1979) redefined the concept of the family *Elasmomycetaceae*, and BEATON *et al.* (1984) considered the anatomy of the hymenophoral trama and spore ornamentation as the main characters to be used. ZHANG & YU (1990) modified the systematics of the three genera reinforcing the importance of the spore ornamentation.

As a result of our own research (CALONGE & PEGLER, 1998; MORENO-ARROYO *et al.* 1998a-b, 1999), we consider that the main taxonomic characters to distinguish the three genera are the following:

- **Peridium**: In previous works, it seems to be considered as a character of minor importance, not withstanding the following comments by earlier authors: "...haud distinctum vel nullum ..." , in *Gymnomyces* (MASSEE & RODWAY, 1898); "...enterely of filamentous hyphae or with scattered sphaerocysts or truly heteromerous in at least the inner layer...", in *Martellia* (SINGER & SMITH, 1960); "...well developed, the epicuticular layer distinctive by virtue of being a trichodermium or at least with numerous dermatocystidia...", in *Zelleromyces* (SINGER & SMITH, 1960). The peridium is almost forgotten by later authors (PEGLER & YOUNG, 1979; BEATON *et al.*, 1984; ZHANG & YU, 1990). As a matter of fact, the peridium may appear homomerous or heteromerous, and with or without sphaerocysts and laticiferous elements in the three genera.

- **Hymenophoral trama**: This is an important character to separate the three genera. In *Gymnomyces* it is heteromerous, with nests of sphaerocysts, while in *Martellia* and *Zelleromyces* there are not nests but some scattered sphaerocysts may be observed (SINGER & SMITH, 1960).

- **Columella and sterile base**: They are variable characters which may be present, absent or sometimes appear rudimentary, according to different species.

- **Spores**: They are variable in size, shape and ornamentation in the three genera making it difficult to establish a criterion, using spore morphology as the sole character, to separate these genera, as suggested by ZHANG & YU (1990). They vary from typically reticulate, as in *Zelleromyces* (Figs. 1a-b); warty or cresty, as in *Gymnomyces* (Figs. 1c-d), to typically warty, as in *Martellia* (Figs. 1e-f).

- **Basidia and sterigmata**: They are of a secondary importance, but of some help to differentiate related species, considering morphology and number of sterigmata on the basidia.

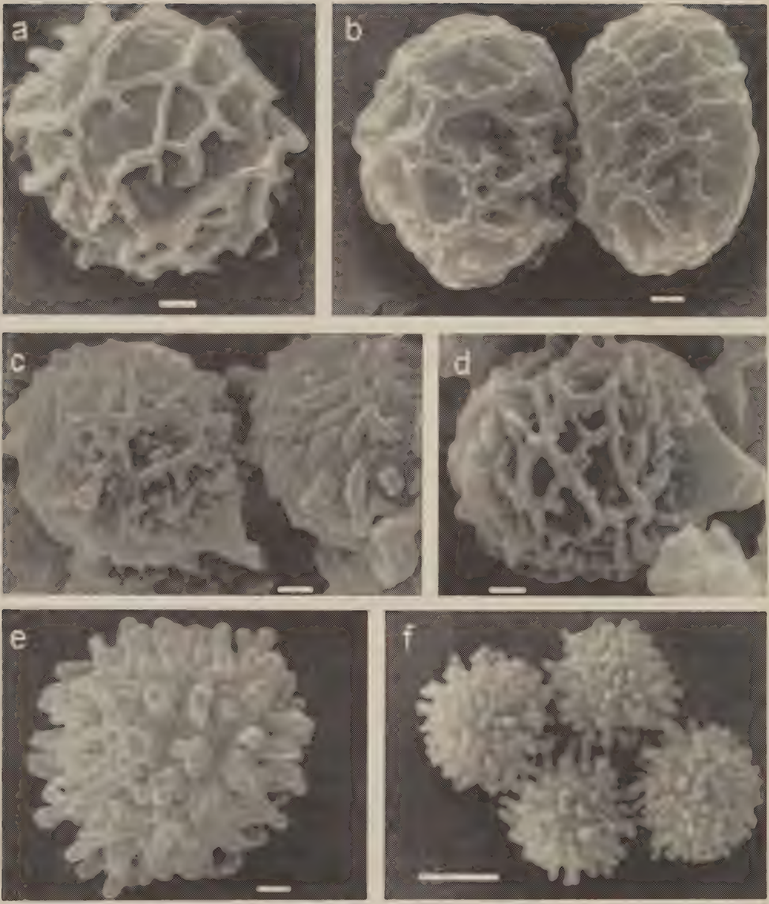


**Fig. 1**. Spores. (a) *Zelleromyces hispanicus*; (b) *Z. giennensis*; (c-d) *Gymnomyces dominguezii; (e-f) Martellia pila.*

- **Cystidia**: Two types of cystidia may be observed in these genera; leptocystidia, which are cylindric, hyaline and thin-walled, and pseudocystidia, fusoid, with oleiferous contents (BEATON *et al.*, 1984). Their presence or absence help in some way to separate genera.

- **Latex, laticiferous and oleiferous hyphae**: We do agree with ZHANG & YU (1990) in their opinion concerning the relative taxonomic value of these characters. SINGER & SMITH (1960) decided to take the presence of laticiferous hyphae producing a copious latex as the main character to separate *Zelleromyces* from *Gymnomyces* and *Martellia*. However, PEGLER & YOUNG (1979) and BEATON *et al.* (1984) argued against this, since the presence or absence of latex rarely can be used to establish relationships among the *Russulales*. On the other hand, THIERS (1984) has observed a drastic variation in latex-production in *Arcangeliella,* depending on the different developmental stages of the basidioma. If the collected basidiomata do not exude any latex, even though they have abundant laticiferous hyphae in peridium and trama, it may be due to aging, immaturity, dryness and so on. On the other hand, specimens belonging to the three genera included in the *Elasmomycetaceae* can develop a latex or laticiferous elements. Are they *Zelleromyces* or not? It is, indeed, difficult to say if we use only this character for the separation of the three genera.

On the other hand, when we observe the anatomy of the *Gymnomyces*-group under the light microscope, both, laticiferous and oleiferous hyphae may be present,. But, how can they be distinguished ?. This is another interesting task.

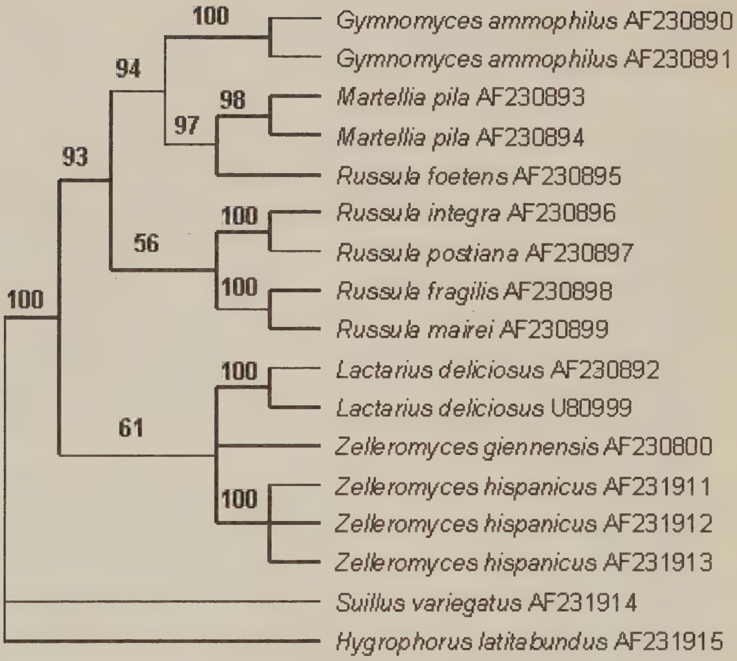
Finally, habitat and ecology could be considered, but they are frequently overlooked. In the case of *Zelleromyces*, for instance, there are several species restricted to *Pinus* spp., such as *Z. cinnabarinus, Z. hispanicus, Z. giennensis,* etc., or to *Eucalyptus* spp., such as *Z. striatus, Z. daucinus, Z. australiensis,* etc., or in mixed forests, like *Z. stephensii, Z. josserandii,* etc.

The DNA sequence of the ITS regions of the rDNA gene helps not only to separate two close species but also to fuse them when the differences are too little. Regarding *Gymnomyces, Martellia* and *Zelleromyces*, we do not know any available publication where this subject is studied, that is why our assays could represent the first application of these new techniques to the clarification of the genetic identity of these three genera.

We performed a cladistic analysis of ITS regions, including the 5.8S rDNA, of the collections mentioned in Table 1 and the sequence of *Lactarius deliciosus* retrieved from the GenBank. Of the 685 characters, 166 were informative (77 in the ITS1 region and 89 in the ITS2 region). Using *Suillus variegatus* and *Hygrophorus latitabundus* as outgroup, the analysis produced three parsimonious trees with a length of 522, a consistency index (CI) of 0.812 and a retention index of (RI) of 0.789, by using PAUP's branch-and-bound algorithm. The strict consensus tree is shown in Fig. 2. The three trees differ in the position of *Zelleromyces giennensis*. It may be either a sister group of the two *Lactarius delicious* or be a sister group of *Lactarius delicious* together with the three collections of *Zelleromyces hispanicus.* Morphological characteristics make the latter alternative most probable.

The monophyletic clade formed by all the ingroup taxa is not suprisingly very well supported (100 % bootstrap). *Martellia* and *Gymnomyces* species are closely related to *Russula* species, and the monophyletic clade of these genera is well supported (93 %). This is in agreement with morphological data.The clade formed by species of these genera is the sister group of the clade in which *Lactarius* and *Zelleromyces* are included; although this last clade is weakly supported (61 %).

HAWKSWORTH *et al.* (1995) retain two families in Russulales: *Elasmomycetacea* for the gasteroid genera and *Russulaceae* for the agaricoid ones. Based on our phylogenetic analysis these two families should not be maintained. Thus, we consider that the family *Elasmomycetaceae* is a synonymy of the *Russulaceae.* Attempts to amplify and sequence the DNA from *Martellia mistiformis* Mattirolo and *Zelleromyces cinnabarinus* Smith & C.A. Brown (type collections from NY and MICH herbaria respectively) are in course.


100
94
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97
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100
100
100
61
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Gymnomyces ammophilus AF230890
Gymnomyces ammophilus AF230891
Martellia pila AF230893
Martellia pila AF230894
Russula foetens AF230895
Russula integra AF230896
Russula postiana AF230897
Russula fragilis AF230898
Russula mairei AF230899
Lactarius deliciosus AF230892
Lactarius deliciosus U80999
Zelleromyces giennensis AF230800
Zelleromyces hispanicus AF231911
Zelleromyces hispanicus AF231912
Zelleromyces hispanicus AF231913
Suillus variegatus AF231914
Hygrophorus latitabundus AF231915

**Fig. 2.** Molecular analysis of the specimens included in Table 1 and *Lactarius deliciosus* retrieved from the Genbank. Strict consensus of three parsimonious trees under branch-and-bound algorithm. Percentages from 1000 bootstrap replications are given on the branches

As a conclusion of our morphological and molecular research we can state as follows:

1.- The family *Elasmomycetaceae* could be a synonym of the *Russulaceae.*

2.- The genera *Gymnomyces* and *Martellia* could well be considered as gasteroid forms of genus *Russula.*

3.- The genus *Zelleromyces* could represent gasteroid forms of the genus *Lactarius.*

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# PHENETIC PLASTICITY IN PLEUROTUS DJAMOR

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**Abstract**: *Pleurotus djamor* has become known for its variability in basidiome color and stature. To quantify the breadth of phenetic plasticity within the species, over 40 $P_1$ collections or dikaryon cultures of putative *P. djamor* from Asia and America were examined. Thirty-two strains were fruited, from which 20 collections were chosen for combined analyses, including phenetic analyses for qualitative and quantitative characters and sexual compatibility tests. Two types of compatibility tests proved all selected collections were compatible, and phenetic analyses showed no significant clustering power using macro- or micromorphological characters. $F_1$ hybrid basidiomata were produced and viable $F_1$ basidiospores obtained. $F_1$ hybrid basidiomata were unpredictable for color, and basidiome color intensity was reduced under "standardized" fruiting conditions. The species appeared to be pantropical, varying within acceptable infraspecific bounds except for basidiome color, which was not linked to geographic origin, other morphological characters, or light intensity during basidiome ontogeny.

Key words. Phenotypic plasticity, phenetic analysis, *Pleurotus*

## INTRODUCTION

The name *Pleurotus djamor* (Fr.) Boedijn represents a widespread, tropical to subtropical taxonomic complex. Distinctions within the complex have been determined primarily using morphological characteristics (Corner, 1981; Pegler, 1977; Singer,

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1986; Guzman *et al.*, 1992). Recent studies of the genus, however, have incorporated intercompatibility tests as additional criteria for taxonomic delimitation (Petersen, 1992, 1995; Petersen and Hughes 1993; Vilgalys and Sun, 1994).

*Pleurotus djamor* was originally described by Rumph (1750) in *Herbarium Amboinense* under the name *Boletus secundus arboreus.* Rumphius described the mushroom as growing from trees: pileus imbricate, circular-oblong to liver-shaped, displaying reddish coloration when young, fading to white, dull-yellow, or yellowish-tan in maturity; stipe short or sometimes lacking. The species epithet was sanctioned by Fries (1821) as *Agaricus djamor* in tribe *Pleurotus,* subtribe *Concharia.* In 1836, Fries transferred the species epithet to *Lentinus,* and Boedijn (1959) transferred the epithet to *Pleurotus.*

Corner (1981) reported on southeast Asian specimens of *P. djamor* as a species expressing wide morphological variability. Basidiomata were described as monomitic when young, becoming dimitic in age, and color, micromorphology and habit were used to propose six varieties, among which intermediates were also noted.

Guzman *et al.* (1992) divided the taxonomic complex into separate species. In their taxonomic scheme *P. djamor* was equated with Corner's (1981) definitions of *P. djamor* var. *djamor* and var. *roseus.* In addition, Corner's (1981) *P. djamor* var. *fuscoroseus* was considered equivalent to *P. ostreatoroseus* Sing. from Brazil and Mexico and with *P. salmoneostramineus* Vassileva from the northern Far East.

**Species Concepts.** - Interpretation of results presented below is dictated by the "species concept" brought to the paper by the reader. Most previous treatments (Corner, 1981; Pegler, 1977; Singer, 1986) have assumed that the term "species" was based on morphological distinctions, with genetic isolation of the various "morphs" untested. Several treatments which have included the *P. djamor* complex, however, have used a species concept based on sexual compatibility (Guzman et al., 1992; Petersen, 1992, 1995; Petersen and Hughes, 1993) with morphological variation subordinate. The morphological species concept for agarics was summarized by Smith (1968), but a wider discussion was written by Kuyper (1988). The intent of the research described here was to ascertain: 1) the relationship of sexual compatibility (and interfertility) to morphological variability in this taxonomic complex; and 2) to offer a preliminary biogeographic range for the biological species.

## MATERIALS AND METHODS

**Culture establishment and storage.** - Specimens and cultures were received or collected in a number of states (i.e. basidiomata, spore prints, dikaryon cultures, etc.), generally paralleling those summarized by Petersen et al (1999) for another project on *Flammulina.* Techniques for establishing mono- and dikaryon cultures have been decribed by Petersen et al. (1999) and Gordon and Petersen (1992). Cultures were stored using the technique by Burdsall and Dorworth (1994).

**Fruiting dikaryotic strains.** - The basic interbreeding unit in compatibility tests is a haploid, monokaryon culture (usually termed the "monokaryon"). In cases where only a dikaryon culture was available, or when no descriptive notes on $P_1$ basidiomata were at hand, dikaryon cultures were fruited to provide descriptive information and monokaryon isolates. This fruiting process generally followed that by Stamets (1993), but is described here: 1) to detail the adjustments made to Stamets' technique; and 2) to furnish a means to duplicate our results.

Spawn. -- A mixture of 200 g rye grain, 200 ml deionized water and one gram powdered calcium sulfate were mixed in a one-quart Mason jar. The lid of the jar was punctured with an ice pick to make 15-20 holes for gas exchange. Lids were lined with cotton to prevent contamination. The jars were then autoclaved for one hour to sterilize the rye medium, and allowed to cool to room temperature in a laminar flow hood.

Dikaryotic mycelium was allowed to colonize an entire malt extract (Difco, 15 gr/l) agar (Difco Bacto 20 gr/l; deionized water 1 l: MEA) Petri plate (60 mm diam). Mycelium grown in the MEA plates was cut into small squares (approx 20) and transferred to the Mason jar of rye medium. Each jar was shaken vigorously to "evenly" disperse the colonized agar pieces in the rye medium for faster and homogeneous colonization. Inoculated jars were placed in a growth chamber in the dark at 24-26° C. After seven days, jars were shaken vigorously to break the mycelium and allow for recolonization ending on day 14.

Fruiting Substrate. -- A wheat-straw substrate was concocted with the following ingredients: 140 g wheat straw (Agrifeed and Pet Supply), 140 g deionized water, 12 g rice bran (EnerG Foods), 6 g powdered calcium sulfate, 1.5 g powdered calcium carbonate. These ingredients were mixed in a polypropylene bag (12 X 24 in) closed with a twisted wire and autoclaved for one hour. The bags were allowed to cool to room temperature in a laminar flow hood.

The mycelium-colonized rye grain (spawn) was poured into the bags of wheat-straw substrate and the bags closed again. The bags were shaken to disperse the spawn throughout the wheat straw substrate, and were placed on a laboratory table top at 22-26° C, with fluorescent room lighting at 8-10 hrs/da. Once fully colonized, the bags were placed under benches on the gravel floor in a greenhouse at 21-26° C, with relative humidity 75-90%. The amount of light received was dependent on outside weather conditions.

Basidiome initiation. -- At the time of placement in the greenhouse, circular holes (1-3 inches diam.) were cut in the polypropylene bags to allow primordium formation and basidioma development. Some isolates fruited within two da while others took up to six wks to form primordia. All collections progressed from primordia to mature basidiomata in 3-7 da with rapid senescence after maturity.

**Terminology.** - In order to interpret results presented below, the following terminology is used: $P_1$ = basidiomata collected from nature, dikaryon cultures established from them or received from other sources (i.e. culture collections, etc.) and single-basidiospore isolates (SBIs) derived from such basidiomata; $F_1$ = dikaryon cultures established from $P_1$ SBI intercollection pairings, basidiomata resulting from such dikaryons, and SBIs derived from $F_1$ basidiomata.

**Phenetic Study.** - Macro- and micromorphological characters that varied in a quantitative or qualitative manner were used in a comparison of phenetic similarity among basidiomata from 20 selected collections. Qualitative characters were recorded as a 1 or 0 indicating presence or absence of the following characters: hygrophanous areas on the pileus, cheilocystidia, pleurocystidia, and various colors. Basidiome colors of $P_1$ and $F_1$ basidiomata were noted according to Kornerup and Wanscher (1978). Colors were grouped into nine classes with each collection given a 1 or 0 depending on whether or not it exhibited a color found in that class. Colors were classified as following: Class 1- white (1A1)); Class 2- yellowish white (2A2), pale yellow(2A3), light yellow(2A4-5); Class 3- yellowish gray (2-3C-D2), grayish yellow (2B-C3-5); Class 4- brownish gray (4-11C-F2), grayish brown (4-11D-F3), yellowish brown (5D-E8), light brown (5-7D4-8), brown (6-7E-F4-5), brownish orange (5-7C5-8); Class 5- orange-gray (5-6B2), grayish orange (5-6B3-6); Class 6- grayish red (7B3-6, 8-10B-C4-5), reddish gray (7-11B2); Class 7- orange-white (5-6A2), light orange (5-6A4-5); Class 8- pinkish white (8-13A2), pastel red (7-10A4), pale red (7-12A3), pink (11-13A4); Class 9- dark brown (6-9F5-6). A spore print on aluminum foil was made from one basidiome of each fruited collection to be used for spore measurements and subsequent viability tests.

The following quantitative characters were measured: basidial length, basidial width, cheilocystidial length, cheilocystidial width, spore length, and spore width. Quantitative variables were entered into a spread sheet (Excel) and a correlation matrix was created (data not shown). No variables had a correlation coefficient of 0.7 or greater, indicating that all variables were independent enough to be used in the analysis. Each variable was tested for normality by creating box plots. Euclidean distances were calculated on a rectangular SYSTAT (1995) file of the following variables: basidial length, basidiospore length, basidiospore width, basidial width (transformed), basidial length (transformed), cheilocystidial length (transformed), and cheilocystidial width (transformed).

Spore length and spore width were examined by measuring twenty spores from each collection. Basidial length and basidial width were examined by measuring fifteen basidia from each collection.Cheilocystidial length and width were examined by measuring fifteen cheilocystidia, being sure to include a broad sample of all shape variants found in that collection.

To combine quantitative characters with qualitative characters in a single data analysis, quantitative characters were converted to character state classes in the following manner: Spore length 8.0-8.99 μm (0); 9.0-9.99 μm (1); >10.0 μm (2); spore width 4.00-4.99 μm (0); >5.00 μm (1); basidia length 20.0-22.99 μm (0); 23.0-24.99 μm (1); >25.0 μm (2); basidial width 5.0-5.99 μm (0); >6.0 μm (1); cheilocystidial length 18.0-19.99 μm (0); 20.0-21.99 μm (1); 22.0-24.99 μm (2); cheilocystidial width 6.0-6.99 μm (0); >7.0 μm (1). These converted quantitative characters were then combined with the qualitative characters in a rectangular SYSTAT file.

All phenetic data were analyzed with the statistical program SYSTAT (1995). Euclidean distances and Goodman-Kruskal gamma coefficients (Goodman and Kruskal, 1954) were calculated and several algorithms were used to create phenetic trees displaying relationships among collections. The algorhithms used included average linkage (UPGMA), single linkage and complete linkage modes

**Reference Collections.** - Six "reference" collections (i.e. exemplars) were selected to represent the range of color morphs found in this complex as well as diverse geographic origins. Geographic distribution of all collections examined in this study is furnished under "Materials and methods" below. Reference collections were: 1) 6346 pink morph, Malaysia; 2) 7444 gray morph, New Zealand; 3) TMI 31760 white morph, Japan; 4) IBUG $7_1$ pink morph, Mexico; 5) 7839 gray morph, Costa Rica; and 6) 3467 white morph, Puerto Rico. Twelve SBIs were paired in all combinations to perform a self-cross for each of these collections (data not shown). All 12 SBIs were assigned mating types based on their reactions with other SBIs. Representatives of each mating type were selected as the four tester strains used in intercollection pairings.

**Sexual incompatibility.** - Intercollection intercompatibility was determined by the ability of paired donor monokaryon isolates to produce mycelium with clamp connections (i.e. dikaryotization). The four tester strains from each of the six reference collections were paired in all possible combinations, resulting in 16 pairings between each of the reference collections.

Twenty-nine non-reference collections were paired with one of the reference collections in 2 X 2 pairings in order to confirm their placement in the taxonomic complex. These collections are summarized below and in Table 1.

**Intercollection interfertility.** - Tester strains of the six reference collections ($P_1$) were paired in all combinations (pairings = 16). Each of these pairing sets resulted in a minimum of 7 and a maximum of 16 dikaryotization events. Four “healthy” dikaryons ($F_1$) from each pairing set (i.e. 4/7-16; total 60 dikaryons) were isolated. In order to assess interfertility, an attempt was made to fruit each of these $F_1$ dikaryons using the fruiting method described above. From $F_1$ basidiomata, $F_1$ SBI germlings were isolated on MEA and checked for clamp connections. In descriptions of $F_1$ hybrids, spore statistics are stated in parentheses after each pairing set: number of germinated spores harvested: number of germlings surviving: number of monokaryons within surviving germlings.

Interfertility was determined by the ability of an intercollection dikaryon ($F_1$) to produce $F_1$ “hybrid” basidiomata with viable $F_1$ spores.

**Material examined.** - Terminology in the list of materials used is as follows: BAS-N = basidiomata from nature; BAS-D = basidiomata preserved (dried) at receipt; BAS-C = basidiomata from commercial source; SPR = spore print; DIK = dikaryon culture. TENN numbers within parentheses = accession numbers of preserved basidiomata in herb. TENN (Holmgren *et al.*, 1981).

AUSTRALIA: New South Wales, vic. Sydney, IX.97, coll. B. Rees, on Mangrove (BAS-D, SPR). COSTA RICA: Prov. Puntarenas, vic. San Isidro de la General, 17.VI.95, coll. RHP, no. 7839 (TENN 53833) (BAS-N); Prov. Puntarenas, vic. San Vito, 17.VI.95, coll. RHP, no. 7840 (TENN 53847) (BAS-N). GUATEMALA: vic Guatemala City, commercial strain courtesy Ruth DeLeon, "RDL 1" (DIK). INDIA: Vic. Puna, date unknown, Agharkar Research Institute Culture Collection, no. MACS-M-287, our no. 10270 (TENN 57765) (DIK, as *P. eous*, see Dighe and Kulkarni, 1998); location and source same, MACS-M-253, our no. 10271 (TENN 57766) (DIK as *P. eous*, see Dighe and Kulkarni, 1998); location and source same, MACS-M-263, our no. 10272 (TENN 57767) (DIK as *P. eous*, see Dighe and Kulkarni, 1998). JAPAN: location and date unknown, Tottori Mycological Institute culture collection, no. TMI

31760 (DIK); same data, TMI 30258 (DIK); location unknown, date unknown, Edible Mushroom Institute, Hokken Sangyo Co., Ltd., ("JAPAN") (BAS-C). MALAYSIA: Location unknown, date unknown, leg. R. Watling (SPR). MEXICO: Est. Chiapas, vic. Tapechula, X.97, coll. Villegas-Rios, no. VR-1786 (DIK); Est. Jalisco, vic. Guadalajara, date unknown, Instituto Biologia culture collection, IBUG-53 (DIK); location same, date unknown, IBUG 7$_2$ (DIK); location same, date unknown, IBUG-3 (DIK); location same, date unknown, IBUG 7$_1$ (DIK; see Cedano et al., 1993); Est. Tabasco, vic. Teapa, Estacion Agronomica UNAM, 20.X.91, coll. RHP, no. 4326 (TENN 50405) (BAS-N); location same, date same, no. 4375 (TENN 50449) (BAS-N); Est. Veracruz, Xalapa, 14.VII.93, coll. A. Estrada-Torres, no. 6275 (TENN 52372) (BAS-N); Est. Veracruz, vic. Catemaco, UNAM Estacion Tuxtlas, 24.X.91, coll. RHP,

## Table 1

Colors exhibited by intercompatible fruited P$_1$ strains of *Pleurotus djamor*

| Group | Collections | Colors exhibited |
|---|---|---|
| 1 | K7018, RDL1, **TMI31760**, IBUG53, IBUG3 | white, yellowish white (2A2-3), yellowish gray (4B2), grayish yellow (4B3) |
| 2 | 4326, 4375, 3467, McAdoo, 6275 **7839** | white, yellowish white (2A2-3),light yellow (4A4), yellowish gray (4B2), grayish yellow (4B5), orange gray (5B2), grayish orange (5B3-4) |
| 3 | 4331, 6278, 6270, 4381, 8013, **3467**, 8013, 441A, 7840, VR1786 | white, yellowish white (2A2-3), yellowish gray (3-4B2), grayish yellow (4B3-4), yellowish brown (5D-E4), grayish brown grayish orange (5B3-4), brownish orange (5C3-4), orange gray (5B2), orange white (5A2), pale orange (5A3), brownish gray (4C2) |
| 4 | PDD88115, 7167 **7444** | yellowish white (2A2-3), orange white (5A2), orange gray (5-6B2), grayish orange (5B3-5), brownish orange (5-6C3-5), brownish gray (5C2), brown (6D-E4-5), grayish brown (5D3, 6-7E3, 7F3), dark brown (6F4-6, 7F4) |
| 5 | TMI30258, **6346**, Japan | grayish orange (6B3-4), orange white (5-6A2), pale orange (6A3), yellowish brown (5E-F4), brownish orange (6C4-5, 7C4), grayish red (7-8B3, 8C3-4), pinkish (7-8A2, 11A2), pale red (10A3), pink (11A3) |
| 6 | **IBUG7$_1$**, IBUG7$_2$, IBUG39, MF1-18, ASM7021, 6346, 10270, 10271, 10272 | white, yellowish white (2A2-3), yellowish gray (4B2), grayish brown (5F3, 6D-E3, 7D3), light brown (6D4), brown (6-7E4), brownish orange (6C3, 5), grayish orange (5-6B4), orange gray (5B2), grayish red (7B5, 8B3), pastel red (7A4), pinkish white (7-11A2), pink (11A3) |

no. 4375 (TENN 50449) (BAS-N). NEW ZEALAND: North Island, Auckland Dist, Waitekere Ranges, 1.VI.94, coll. RHP, no. 7167 (TENN 54051) (BAS-N); North Island, Omahuta Forest, 5.VI.94, coll. A.S. Methven, no. 7444 (TENN 54064) (BAS-N); North Island, Auckland Dist., Auckland Airport, on *Coryline australis*, 12.II.88, coll. P. Buchanan, no. PDD 88-115 (DIK, see Rees-George *et al.*, 1990). UNITED STATES: Florida, Sarasota Co., Myakka River State Park, 3.VII.93, coll. R. Williams, no. 6278 (TENN 52370) (BAS-D, SPR); Sarasota Co., Myakka River State Park, 5.VII.93, coll. R. Williams, Williams no. 441A (BAS-D, SPR); Puerto Rico, Forestal Preserve Rio Abajo, 5.XII.90, coll. RHP & S.A. Gordon, no. 3467 (TENN 49017) (BAS-N); Texas, vic. San Antonio, date unknown, coll. A.S. Methven, Methven no. ASM 7021 (Methven herbarium, Eastern Illinois University) (SPR). VENEZUELA: Location and date unknown, coll. T. Iturriaga (BAS-D, SPR).

## RESULTS

**Establishment of biological species coherence.** - Inter-reference-collection grid. -- Figure 1 shows the experimental design of inter-reference-collection pairings (n = 16). In all cases, reference collections were intercompatible, although some individual monokaryon isolates (notably those of collection 6346) did not dikaryotize their opposite strain.

"Recognition grid—" Reference collection ($P_1$) SBIs were compatible with random SBIs of other $P_1$ collections of the same putative biological species (Table II). Results of inter-reference-collection pairings and "recognition grid" pairings resulted in 30 collections considered part of the shared gene pool, or biological species.

**Mating system.** - SBIs from all six reference collections were subjected to self-cross experiments. All proved to be tetrapolar (data not shown).

**Macromorphology.** - Basidioma color -- In some instances, $P_1$ basidiomata were annotated when collected, so base-line color notes were available. Nonetheless, $P_1$ dikaryon cultures were fruited for all experimental strains. Under "standard" greenhouse fruiting conditions, pileus colors were almost universally damped. Although color variation was easily detectable, only $P_1$ strains with white or off-white basidiomata retained their original color when fruited. The three color categories described by Petersen (1995) could not be maintained and in several instances of $P_1$ clonal basidiomata more than one color category was exhibited.

For purposes of phenetic analysis, fruited basidiomata were grouped according to the color ranges they displayed (Table 1). Basidiomata of each collection within a color group exhibited most, but not necessarily all, of the colors ascribed to that group. These groups overlapped and therefore were not discrete.

Changes in basidiome color routinely occurred during basidiome development. For example, basidiomata of collection 6346 were pink in the primordial stage but faded through orange-white becoming yellow-white to off-white by maturity, but later fruiting flushes from the same bag remained pink through all ontogenetic stages. Basidiomata of collection ASM 7021 were pink, pale red with brownish gray to grayish brown pubescence in the primordial and young stage, fading to orange white, yellow-white with orange-grey pubescence by maturity. Basidiomata of other collections, such as members of group four, were dark brown in the primordium and young cap stage, a color not covered by any of the original morphotype groupings.

**Phenetic study.** - Mean measurements of micromorphological structures over all collections were as follows: spore length 8.01-10.53 µm; spore width 4.36-5.15 µm; basidial length 20.69-26.64 µm; basidial width 5.83-6.84 µm; cheilocystidial length 18.14-24.36 µm; cheilocystidial width 6.58-8.78 µm.

Visual assessment of box plots (unpubl. data) indicated that spore length and basidial length were normally distributed. Basidial width, cheilocystidial length, cheilocystidial width, and spore width showed skewed distributions indicating departure from normality. Basidial width was normalized by a hyperbolic tangent transformation. Cheilocystidial length was sin transformed, cheilocystidial width was arc tangent transformed, and spore width was hyperbolic tangent transformed resulting in a normal distribution for each variable. Normality was examined for transformed variables by visual assessment of box plots.

Data analysis. -- A join (agglomerative) single linkage clustering algorithm was used to create a tree of phenetic relationships of quantitative variables (phenogram not shown). Euclidean distances between clusters ranged from 0.1628 (7839 and RDL1) to 0.5938 between a cluster with TMI 31760 and IBUG $7_1$ and a cluster containing the other 18 collections. Four major subclusters were created: 1) TMI 31760, IBUG $7_1$, TMI 30258; 2) 3467, 7839, RDL1, 6275, 6346, JAPAN, MCADOO; 3) K7018, ASM 7021, 441A, 4375 7840; and 4) 4331, 7167, 7444, IBUG 39, PDD 88115.

A complete linkage clustering algorithm was used to create a phenogram using quantitative variables (phenogram not shown). Euclidean distances between clusters ranged from 0.1628 (7839 and RDL1) to 2.3645 between a cluster of seven collections (top of tree) and 13 collections (bottom of tree). These two clusters were then each divided into two major clusters creating four distinct subclusters: 1) TMI 30258, IBUG $7_1$, TMI 31760; 2) MCADOO, JAPAN, 6346, 6275; 3) 3467, 7839, RDL1, 4375, 441A, ASM 7021, K7018; 4) PDD 88115, IBUG 39, 7444, 7167, 4331, 7840.

An average linkage clustering technique was applied to the data creating a phenogram of quantitative variables (see Fig. 1). Euclidean distances between clusters ranged from 0.1628 (7839 and RDL1) to 1.46 between a cluster of seven collections (top of tree) and 13 collections (bottom of tree). These two clusters were each divided into two major clusters creating four subclusters each separating at distances around 0.45-0.66: 1) TMI 30258, IBUG $7_1$, TMI 31760; 2) MCADOO, JAPAN, 6346, 6275; 3) 3467, 7839, RDL1, 4375, 441A, ASM 7021, K7018; 4) PDD 88115, IBUG 39, 7444, 7167, 4331, 7840.

Phenotypic distances were calculated using Gamma coefficients on qualitative variables. A phenogram was created using the average linkage method (phenogram not shown). Distances ranged from 0.0 to 1.28. The collections were grouped into three major clusters with little to no separation of collections within these clusters: 1) 6346, K7018, RDL1, TMI 31760, IBUG $7_1$, IBUG 39, 4375, 3467, MCADOO, 6275, 4331, 441A, 7840, and 7839; 2) TMI 30258, JAPAN; and 3) ASM 7021, 7167, 7444, PDD

88115. This phenogram showed little separation between collections indicating low resolution, and further analyses are not shown here.

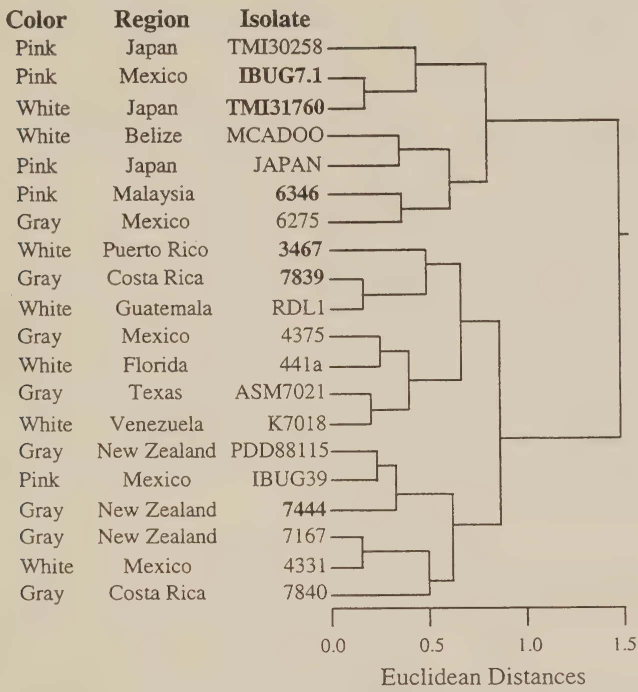

Fig. 1. Phenetic tree showing relationships among collections of *Pleurotus djamor*, created using an average linkage clustering algorithm of Euclidean distances of only quantitative variables. Reference collections in boldface.

A single linkage algorithm was applied to the data to create a phenogram combining quantitative and qualitative variables (phenogram not shown). Distances ranged from 0.0 to 0.46 indicating little resolution. Three major clusters could be discerned: 1) JAPAN, TMI30258; 2) 6346, IBUG $7_1$, MCADOO, TMI 31760, 441A, 3467, 4331, 7840, 7839, 4375, K7018, RDL1, IBUG 39, 6275, PDD 88115, 7167, 7444; 3) ASM 7021.

Gamma coefficients were calculated and a join-cluster algorithm using the average linkage method was used to create a phenogram combining quantitative and qualitative variables (phenogram not shown). Distances ranged from 0.0 to 1.06 with five major clusters separating from each other at a distance of 0.5 or greater: 1) ASM 7021; 2) PDD 88115, 7167, 7444; 3) 4331, 7840, 7839, 6275, 3467, 441A, TMI 31760, MCADOO, RDL1, K7018, IBUG 39, 4375; 4) IBUG $7_1$ and 6346; 5) TMI 30258 and JAPAN.

A complete linkage algorithm was also used to create a phenogram combining quantitative and qualitative variables (see Fig. 8). Distances ranged from 0.0 to 1.97. Two major clusters were separated at a distance of 1.97: 1) 7444, 7167, PDD 88115, ASM 7021; and 2) the sixteen remaining isolates. This second cluster could be subdivided into two subclusters separating at a distance of 1.14: 1) 6346, IBUG $7_1$, TMI 30258, JAPAN; and 2) MCADOO, TMI 31760, 441A, 3467, 6275, 7839, 7840, 4331, 4375, IBUG 39, K7018, RDL1.

**Intercollection interfertility.** - Careful notes on basidiome color were made for $F_1$ hybrid basidiomata. Reference to colors from Kornerup and Wanscher (1973) require the reader to consult that volume directly.

**$F_1$ hybrid basidiomata between 6346 X 7839.**

6346:8 X 7839:12 - pastel red (7-10A4), pale red (7-12A3), pinkish white (8-13A2), fading to orange-white (5-6A2), pale orange (5-6A3), yellowish white (2A2), white (20/17/16)

6346:8 X 7839:2 - pinkish white (8-13A2) to orange-white (5-6A2), grayish orange (5-6B3-6), yellowish white (2A2), white (20/20/19)

6346:11 X 7839:5 - pastel red (7-10A4), pale red (7-12A3), pinkish white (8-13A2), orange-white (5-6A2), grayish red (7B3-6, 8-10B-C4-5), brownish orange (5-7C5-8), grayish orange (5-6B3-6), yellowish white (2A2) (20/16/12)

6346:5 X 7839:2 - white, yellowish white (2A2), pale yellow (2A3), yellowish gray (2-3C-D2), grayish yellow (2B-C3-5), orange-gray (5-6B2), grayish orange (5-6B3-6) (20/20/18).

**$F_1$ hybrid basidiomata between 7444 X 7839.**

7444:14 X 7839:12 - brownish orange (5-7C5-8), grayish orange (5-6B3-6), grayish brown (4-11D-F3), yellowish white (2A20, white (20/5/4): no hygrophanous areas

7444:15 X 7839:6 - light brown (5-7D4-8), brownish orange (5-7C5-8), grayish orange (5-6B3-6), orange-gray (5-6B2) , yellowish white (2A2), white (20/10/8): pileus margin hygrophanous in age

7444:15 X 7839:2 - brownish orange (5-7C5-8), grayish orange (5-6B3-6), orange-gray (5-6B2), yellowish white (2A2)(20/17/14):pileus margin hygrophanous in age.

**$F_1$ hybrid basidiomata between 3467 X 7839.**

3467:7 X 7839:5 - white, yellowish white (2A2), grayish yellow (2B-C3-5), grayish orange (5-6B3-6), grayish brown (4-11D-F3)(20/18/17)

3467:2 X 7839:12 - orange-gray (5-6B2), brownish orange (5-7C5-8), yellowish white (2A2), yellowish gray (2-3C-D2)(20/19/19)

3467:8 X 7839:2 white, yellowish white (2A2), yellowish gray (2-3C-D2), grayish yellow (2B-C3-5)(20/19/18).

**$F_1$ hybrid basidiomata between TMI 31760 X 7839.**

TMI 31760:9 X 7839:2 - white, yellowish white (2A2), orange-gray (5-6B2), grayish orange (5-6B3-6)(20/20/17)

TMI 31760:7 X 7839:2 - white, yellowish white (2A2), yellowish gray (2-3C-D2), grayish orange(5-6B3-6), orange-gray (5-6B2), brownish orange (5-7C5-8), grayish brown (4-11D-F3)(20/20/20)

TMI 31760:4 X 7839:12 - orange-gray (5-6B2), grayish orange (5-6B3-6), yellowish gray (2-3C-D2), yellowish white (2A2), white (20/20/20)

TMI 31760:4 X 7839:5 – brown 6-7E-F4-5), grayish brown (4-11D-F3), brownish orange (5-7C5-8), grayish orange (5-6B3-6), yellowish gray (2-3C-D2), yellowish white (2A2)(20/20/20).

**$F_1$ hybrid basidiomata between IBUG $7_1$ X 7839.**

IBUG $7_1$:20 X 7839:2 - orange-gray (5-6B2), grayish orange (5-6B3-6), brownish orange (5-7C5-8), yellowish gray (2-3C-D2), yellowish white (2A2), pale yellow (2A3), white (20/20/14)

IBUG $7_1$:7 X 7839:6 - white, orange-gray (5-6B2), grayish orange (5-6B3-6), yellowish white (2A2) (20/20/16).

**$F_1$ hybrid basidiomata between 3467 X 6346.**

3467:7 X 6346:11 - pinkish white (8-13A2), brownish orange (5-7C5-8), grayish brown (4-11D-F3), orange-white (5-6A2), grayish orange (5-6B3-6), yellowish white (2A2)(20/20/20)

3467:2 X 6346:8 - pale red (7-12A3), pinkish white (8-13A2), orange-white (5-6A2), yellowish white (2A2), white (20/20/20)

3467:8 X 6346:8 - white, pinkish white (8-13A2), pale red (7-12A3), pastel red (7-10A4), reddish gray (5-11B2), orange-white (5-6A3), pale orange (5-6A3), orange-gray (5-6B2), yellowish white (2A2)(20/20/19).

**$F_1$ hybrid basidiomata between IBUG $7_1$ X 6346.**

IBUG $7_1$:20 X 6346:8 - pinkish white (8-13A2), pale red (7-12A3), pale orange (5-6A3), orange-white (5-6A2), grayish red (7B3-6, 8-10B-C4-5), brownish orange (5-7C5-8), white (20/20/19)

IBUG $7_1$:11 X 6346:8 - pale red (7-12A3), pinkish white (8-13A2), orange-white (5-6A2), yellowish white (2A2), white (20/20/20)

IBUG $7_1$:2 X 6346:8 - pastel red (7-10A4), pale red (7-12A3), pinkish white (8-13A2), grayish red (7B3-6, 8-10B-C4-5), brownish orange (5-7C5-8), grayish orange (5-6B3-6)(20/20/18)

IBUG $7_1$:7 X 6346:5 - white, orange-gray (5-6B2), pale yellow (2A3), yellowish white (2A2), yellowish gray (2-3C-D2), grayish yellow (2B-C3-5)(20/20/18).

**$F_1$ hybrid basidiomata between TMI 31760 X 6346.**

TMI 31760:4 X 6346:8 - pinkish white (5-13A2), pale red (7-12A3), orange-gray (5-6B2), grayish orange (5-6B3-6), brownish orange (5-7C5-8), pale orange (5-6A3), orange-white (5-6A2), white (20/20/20)

TMI 31760:4 X 6346:5 - orange-gray (5-6B2), grayish orange (5-6B3-6), orange-white (5-6A2), yellowish white (2A2), white (20/20/20)

TMI 31760:6 X 6346:8 - pinkish white (8-13A2), orange-white (5-6A2), yellowish gray (2-3C-D2), yellowish white (2A2), white (20/13/13).

**$F_1$ hybrid basidiomata between 7444 X 6346.**

7444:8 X 6346:5 - dark brown (6-9F5-6), grayish brown (4-11D-F3), brownish orange (5-7C5-8), grayish orange (5-6B3-6), orange-gray (5-6B2), orange-white (5-6A2), yellowish white (20/13/11): pileus streaked hygrophanous in age

7444:14 X 6346:5 – brown (6-9F5-6), light brown (5-7D4-8), grayish brown (4-11D-F3), brownish orange (5-7C5-8), orange-gray (5-6B2), grayish orange (5-6B3-6), grayish yellow (2B-C3-5), yellowish white (2A2), white (20/18/17): no hygrophanous areas

7444:15 X 6346:5- dark brown (6-9F5-6), brown (6-7E-F4-5), grayish brown (4-11D-F3), yellowish brown (5D-E8), brownish orange (5-7C5-8), grayish orange (5-6B3-6), yellowish gray (2-3C-D2), yellowish white (2A2), white (20/16/15): pileus margin hygrophanous by maturity.

**$F_1$ hybrid basidiomata between TMI 31760 X IBUF $7_1$.**

TMI 31760:4 X IBUG $7_1$:7 - brownish gray (4-11C-F2), grayish brown (4-11D-F3), orange-gray (5-6B2), grayish orange (5-6B3-6), yellowish whit (2A2)e, yellowish gray (2-3C-D2), white (20/17/15)

TMI 31760:4 X IBUG $7_1$:11 - orange-gray (5-6B2), grayish orange (5-6B3-6), yellowish gray (2-3C-D2), yellowish white (2A2), white (20/20/18)

TMI 31760:7 X IBUG $7_1$:20 - white, yellowish white (2A2), grayish orange (5-6B3-6)(20/20/17).

**$F_1$ hybrid basidiomata between 3467 X IBUG $7_1$.**

3467:8 X IBUG $7_1$:11 - orange-gray (5-6B2), grayish orange (5-6B3-6), grayish brown (4-11D-F3), yellowish white (2A2), pale yellow (2A3), yellowish gray (2-3C-D2), grayish yellow (2B-C3-5), white (20/?/?)

3467:8 X IBUG $7_1$:20 - orange-gray (5-6B2), grayish orange (5-6B3-6), brownish gray (4-11C-F2), yellowish gray (2-3C-D2), yellowish white (2A2), white (20/19/17)

3467:7 X IBUG $7_1$:7 - grayish orange (5-6B3-6), grayish brown (4-11D-F3), orange-gray (5-6B2), yellowish gray (2-3C-D2), yellowish white (2A2), white (20/?/?)

3467:6 X 7444:20 - brownish orange (5-7C5-8), brownish gray (4-11C-F2), grayish brown (4-11D-F3), orange-white (5-6A2), yellowish gray (2-3C-D2), yellowish white (2A2)(20/20/17).

**$F_1$ hybrid basidiomata between 7444 X IBUG $7_1$**

7444:13 X IBUG $7_1$:11 – brown (6-7E-F4-5), grayish brown (4-11D-F3), brownish orange (5-7C5-8), grayish orange (5-6B3-6), orange-gray (5-6B2), yellowish white (2A2), white, (20/17/17); pileus margin hygrophanous in age.

**$F_1$ hybrid basidiomata between 7444 X 3467**.

7444:8 X 3467:11 – brown (5E-F4, 6-7E-F4-5), grayish brown (4-11D-F3), brownish orange (5-7C5-8), grayish orange (5-6B3-6), orange-gray (5-6B2), orange-white (5-6A2), pale orange (5-6A3), yellowish white (2A2), white (20/15/14)

7444:15 X 3467:7 – brown (6-7E-F4-5), grayish brown (4-11D-F3), light brown (5-7D4-8), brownish orange (5-7C5-8), grayish orange(5-6B3-6), orange-gray (5-6B2), light orange (5-6A4-5), yellowish gray (2-3C-D2), yellowish white (2A2)(20/9/9)

7444:8 X 3467:6 - grayish brown (4-11D-F3), brownish orange (5-7C5-8), orange-gray (5-6B2), pale orange (5-6A3), orange-white (5-6A2), yellowish gray (2-3C-D2), yellowish white (2A2), white (20/14/14): pileus margin hygrophanous in age for all.

**$F_1$ hybrid basidiomata between TMI 31760 X 3467.**

TMI 31760:7 X 3467:11 - grayish brown (4-11D-F3), brownish orange (5-7C5-8), grayish orange (5-6B3-6), orange-gray (5-6B2), yellowish white (2A2), white (20/18/18)

TMI 31760:19 X 3467:11 - white, yellowish gray (2-3C-D2), yellowish white (2A2)(20/19/18); TMI 31760:4 X 3467:7 - grayish brown (4-11D-F3), brownish orange (5-7C5-8), orange-gray (5-6B2), grayish orange (5-6B3-6), yellowish gray (2-3C-D2), yellowish white (2A2), white (20/19/19)

TMI 31760:19 X 3467:7 - grayish brown (4-11D-F3), brownish gray (5-7C5-8), orange-gray (5-6B2), yellowish white (2A2), white (20/20/19).

**$F_1$ hybrid basidiomata between TMI 31760 X 7444.**

TMI 31760:4 X 7444:15 - dark brown (6-9F5-6), yellowish brown (5D-E8), grayish brown (4-11D-F3), brownish orange (5-7C5-8), grayish orange (5-6B3-6), orange-gray (5-6B2), grayish yellow (2B-C3-5), yellowish white (2A2)(20/17/16)

TMI 31760:7 X 7444:8 - dark brown (6-9F5-6), grayish brown (4-11D-F3), brownish orange (5-7C5-8), grayish orange (5-6B3-6), orange-gray (5-6B2), yellowish gray (2-3C-D2), yellowish white (2A2), white (20/15/13)

TMI 31760:19 X 7444:15 - dark brown (6-9F5-6), brown (5E-F4, 6-7E-F4-5), light brown (5-7D4-8), yellowish brown (5D-E8), grayish brown (4-11D-F3), grayish orange (5-6B3-6), orange-gray (5-6B2), yellowish-white (2A2)(20/17/16)

TMI 31760:19 X 7444:8 - dark brown (6-9F5-6), brown (5E-F4, 6-7E-F4-5), grayish brown (4-11D-F3), grayish orange (5-6B3-6), brownish orange (5-7C5-8), orange-gray (5-6B2), orange-white (5-6A2), yellowish white (2A2), grayish orange (5-6B3-6)(20/14/14): pileus margin hygrophanous by maturity in all.

## DISCUSSION

**Morphology versus compatibility.** - It was not the aim of this project to secure the material used to a single specific epithet. From credible literature (Fries, 1821; Corner, 1981), *Pleurotus djamor* seemed to match the description we furnish, in spite of literature to the contrary (i.e. reports of monomitic pileus and stipe flesh). Material was received under several names, of which the most priorable is *Agaricus djamor*, which is also protected as sanctioned by Fries (1821). Because the project dealt with phenetic plasticity judged by fruiting under "standardized" conditions, no preserved type specimen could be guaranteed to produce identical cultures compatible with the same battery of auxiliary collections. Type specimens, especially old types, exhibit some basidiome morphological characters, which either match or do not match morphological characters of basidiomata gathered or produced for this project. Because most type specimens cannot be represented by *ex typus* cultures, there always remains a small disconnect between a type specimen and modern collections which can be subjected to interbreeding experiments. This disconnect may be diminished by comparison of DNA from the type and ancillary specimens, but often DNA extraction from type specimens is impractical or unsuccessful. Thus true identity of the genetic complex described here remains in doubt, awaiting neotypification (and perhaps epitypification) of *Agaricus djamor* and a correct list of synonymous epithets requires identical study for each basionym.

Instead, in order to measure phenetic parameters, care was taken to make sure that all collections used belonged to a single, potentially interbreeding unit. Sexual recognition was eliminated and interfertility diminished as variables, leaving geographic separation (i.e. oceans) as the only obvious barrier to interbreeding in nature. No variability in phenetic characters could be attributed to genetic hiatuses among the various "morphs" in the project.

**Macromorphological basidiome variation.** - Basidiome color. -- Fruitings under "standardized" conditions produced pileus colors that were extremely variable among collections and often within a single collection (see Table IV). Some $P_1$ collections fell within one of the major color classes: TMI 31760, K7018, RDL1 and IBUG 53 fell within the white-tan color morph. Other collections such as 6346 and ASM 7021 spanned two or three color categories. Basidiomata of collection 6346 began as pinkish white, pastel red, to reddish gray, but faded to orange-white, and became yellowish gray to yellowish white by maturity: a second flush of basidiomata from the same bag remained pastel red to pinkish white through maturity. Collection ASM 7021 was pink to pastel red with grayish brown to brownish gray pubescence on the primordia and young basidiomata, fading to orange-white, yellow-white with orange-gray pubescence by maturity: later flushes from the same bag never exhibited pink colors. Finally, basidiomata of three New Zealand collections all showed a lack of hygrophanous areas on the pileus and brown primordial color. Color morphology, overall, was extremely variable even under controlled fruiting conditions, raising doubts about species rank epithets in this complex based on basidiome color.

Our results align closely with Corner's (1981) commentary concerning the *P. djamor* complex from Malaysia. He proposed six varieties based on color, micromorphology, and habit as follows: 1) var. *djamor*; basidiome white to pallid ochraceous, pileus laterally attached; 2) var. *cyathiformis*; basidiome white to off-white, pileus eccentric; 3) var. *terricolus*; basidiome white to off-white, pileus merismatoid, habit terricolous; 4) var. *roseus*; basidiome pink; 5) var. *fuscoroseus*; pileus livid fuscous, lamellae pink; and 6) var. *fuscopruinosus*; pileus fuscous, lamella edge pruinose, umber. Corner (1981) also mentioned intermediates among all these varieties. In our controlled fruitings, the only variant not observed was the pruinose, marginate lamella. Moreover, some $P_1$ basidiomata displayed colors which spanned more than one of Corner's varieties.

Murakami and Takemaru (1990) found that a wild collection of *P. salmoneostramineus* occasionally formed albino basidiocarps when fruited in the laboratory. When the albino form was isolated and crossed with the typical pink morph, $F_1$ offspring ratios indicated that the albinism was governed by a single recessive mutation. This called into question separation of *P. salmoneostramineus* from *P. djamor* at species rank.

**Micromorphological basidiome characters.** - Miticity. -- Understanding of the hyphal construction of stipe and pileus flesh (see Corner, 1953) is essential in placing basidiomata infragenerically. The *P. djamor* complex shares dimitic construction with a group of centrally stipitate taxa (i.e. *P. dryinus, P. levis*), while *P. ostreatus* and similar species exhibit strictly monomitic construction. It is confusing, therefore, when construction in *P. djamor* has been described as both monomitic (Pegler and Heinemann, 1972; Pegler, 1977; Singer, 1986) and dimitic (Corner 1981; Pegler 1986;

Guzman *et al.*, 1992, 1995; Cedano *et al.*, 1993). Other authors, notably Singer (1961), did not mention hyphal construction.

Hymenophoral trama. -- The hymenophoral trama has been reported as dimitic, irregular (interwoven), with hyaline, clamped, generative hyphae and unbranched skeletal hyphae (Singer, 1961; Pegler, 1986; Guzman *et al.*, 1992, 1995; Cedano *et al.*, 1993; Corner, 1981). Pegler and Heinemann (1972) and Pegler (1977) also described the hymenophoral trama as irregular but with only clamped generative hyphae. Our study found the hymenophoral trama to be dimitic and irregular except in very young specimens in which it was monomitic.

Cystidia. -- Most workers (Singer, 1961; Pegler and Heinemann, 1972; Pegler, 1977, 1986; Corner, 1981; Guzman *et al.*, 1992; Cedano *et al.*, 1993) have described the highly variable cheilocystidia as subclaviform, subcylindric, subspheropedunculate, subventricose, sometimes with irregular constrictions, frequently mucronate or capitate, occasionally with an appendix up to 13 µm long, or apical mucilaginous halo or drop. Guzman *et al.* (1995) simply defined cheilocystidia as widely claviform for the two pink “varieties” of *P. djamor*. In our study, cheilocystidia were observed as extremely variable in size and shape, more or less as previously reported.

Traditionally, pleurocystidia were not described, perhaps indicating they were not observed, or explicitly reported as absent (Singer, 1961; Pegler and Heinemann, 1972; Pegler, 1977, 1988; Corner, 1981; Guzman, 1992). Cedano *et al.* (1993) stated that pleurocystidia were present in only one specimen, and were subventricose, clavate, mucronate or with an appendix 4-8.8 X 0.8-2 µm. Guzman *et al.* (1995) reported absence of pleurocystidia, but described "cystidioid elements" as 14.4-20(-26.4) X (4-)4.8-6.4 µm, ventricose, mucronate or lecythiform, frequently with an apical mucilaginous drop, common on sides of gills. Pleurocystidia were found in our study in many collections from diverse geographic areas. They varied from clavate to ventricose, ventricose-rostrate, mucronate or often capitate.

Basidiospores. -- Basidiospores have been described consistently as thin-walled, inamyloid, hyaline, oblong-elliptic to subcylindric with well-defined hiler appendix (Singer, 1961; Pegler and Heinemann, 1972; Corner, 1981; Pegler, 1977, 1986; Guzman *et al.*, 1992, 1995; Cedano *et al.*, 1993). Cedano *et al.* (1993) reported the largest range in basidiospore dimensions: (5.6-)7.2-10.4(-11.2) X 3.2-4(-4.8) µm. A large dimension range was also observed in our study with spores on average measuring 6.84-13.32 X 3.6-5.76 µm. Basidiospore dimensions varied widely within collections from all regions.

**Statistical analyses of taxonomic characters.** - Quantitative character analyses (Fig. 1). -- Superficially, basidiomata of the collections selected for complete analysis were separable by color, and reference collections were chosen, in part, on this distinction. Concommitantly, analysis of some quantitative measurements might have revealed population clusters intuitively unrecognizable. When quantitative characters were subjected to statistical analysis: 1) all proved to be statistically independent; and 2) none provided taxonomically informative collection clusters. Biogeographic distribution, for example, remained relatively random in all phenograms.

Qualitative character analysis. -- Qualitative characters were treated as though present (1) or absent (0), eliminating some further qualitative judgements (i.e. such modifiers as: rare, occasional, common, abundant, weak, strong, etc.). While some clustering could be detected by geographic origin, the phenograms showed little resolution, and no further analysis was performed.

Character combination. -- Qualitative and quantitative character sets were combined in statistical analyses using single, average and complete linkage algorithms. The average linkage method created a tree with five major branches: 1) only a single North American collection; 2) only New Zealand collections; 3) collections from the New World tropics and one collection from Japan; 4) one collection from Mexico and one from Malaysia; and 5) two collections from Japan. Although there appeared to be some grouping by geographic origin (New Zealand and Japan) again it was not consistent with a New World (IBUG $7_1$) and Asian (6346) collection also being most phenetically similar. Using single linkage, collections shifted, with many New World collections now clustered with New Zealand collections. ASM 7021 (Texas) diverged distantly from all other collections, while two clusters remained the same as in average linkage analysis ("JAPAN," TMI 30258; 6346, IBUG $7_1$). The complete linkage algorithm phenogram was almost identical to the average linkage tree, differing mainly in distances at which clusters diverged. The large differences between the single and average linkage trees indicated significant "noise" in the data, with collections displaying much overall phenetic similarity. The large number of color characters may have overwhelmed the analysis, creating trees heavily but not entirely determined by pileus coloration. It must also be noted that some information was lost by converting quantitative characters into qualitative ones. For example, a collection with mean spore length of 8.9 µm was grouped with collections with mean spore length of 8.0-9.0 µm while it was actually more similar to a collection with a mean spore length of 9.1 µm.

Overall there appeared to be a high amount of morphological phenetic similarity and overlap among collections in all of these analyses (quantitative, qualitative, and combined). The New Zealand collections clustered together in all analyses although not always discretely. This indicated that these collections were very similar to each other and may have represented a separate group. In addition, they shared the exclusive characteristics of the absence of hygrophanous areas on the cap and dark brown coloration when young. However, there was much phenetic similarity with other collections and because only three New Zealand collections were available it would be unwise to make definitive statements about their particular status until more collections are examined. Other groupings by major geographic area (see Fig. 4) also indicate that geographic barriers (e.g., oceans) may have prohibited these populations from interbreeding with other continental populations in nature, thus preventing one large panpatric taxon. If this was true then character variation was high and mostly overlapping, making morphological differentiation difficult to discern. If these populations have been isolated since continents separated then morphological characters have not directly diverged under the influence of genetic drift, selection, mutation or other evolutionary factors. The other possibility is that these populations are part of one large panpatric taxon that performs long-distance viable basidiospore dispersal or interbreeds through influence of human intervention (i.e., cultivation).

**Sexual compatibility.** - General caveats. -- SBIs of *P. djamor* are not vigorous for prolonged periods of time. Repeated subculturing of SBIs, necessary to produce sufficient mycelium for pairings, often caused growth to slow and sometimes to effectively stop. Repeated use of the same SBIs was difficult to accomplish across all necessary collections, because new inoculum frequently had to be re-established from the storage culture collection. Pairings between slow-growing SBIs often were incompatible while those of vigorously growing SBIs from the same collection were compatible. Moreover, cultures of the tropical *P. djamor* usually expired when stored under refrigeration.

A second possible reason for occasional incompatible pairings may be shared mating type genes (i.e. common-A, common-B). Identification of this event was not a priority in this study and was not pursued.

Inter-reference-collection pairings. -- Other than occasional strain-specific phenomena, overall it appeared that reference collections were universally intercompatible.

"Recognition grid." -- SBIs of other putative *P. djamor* collections were paired with SBIs from one of the reference collections to assess their potential inclusion in this intercompatibility group (Table II. The following collections exhibited full compatibility with one of the reference collections: RDL1, 6275, 6270, K7018, IBUG 3, MCADOO, 7840, 4326, IBUG53, 4375, 4331, ASM 7021, 7167, 8013, PDD 88115, Villegas-Rios 1786, JAPAN, TMI 30258, MF1-18, 441A, 10270, 10271, 10272, and 6278. This intercompatibility, combined with overall macro- and micromorphology, indicated that these collections were members of the *P. djamor* complex.

Although incompatible with reference collection SBIs, IBUG 39 was successfully fruited and basidiomata were macro- and micromorphologically congruent with those of other collections from the *P. djamor* intercompatibility group. Monokaryons of IBUG 39 were originally isolated using neohaploidization (Petersen, 1992), and were extremely slow-growing with much hyphal lysis, indicating that they may not have behaved “normally” when paired with other SBIs.

Interfertility and $F_1$ hybrid progeny. -- Interfertility tests were performed among all $P_1$ reference collections. $F_1$ dikaryons were successfully fruited and $F_1$ basidiomata produced viable basidiospores which germinated and generally the germlings grew normally, indicating that not only were these $P_1$ reference collections compatible, but were successfully interfertile.

$F_1$ hybrid basidiome color morphology was as variable as the parental reference collections that produced them (see Results).

$F_1$ basidiospore germling survival after germination appeared to be lower in some $F_1$ hybrids between reference collection 7444 and other reference collections. Of 20 $F_1$ SBIs harvested from 7444:14 X 7839:12, four survived; 7444:15 X 7839:6, eight survived; 7444:15 X 7839:2, 14 survived. Other inter-reference-collection pairings usually showed better SBI germling survival. For example, in the four pairing sets of TMI 31760 X 7839, 17, 20, 20 and 20 SBIs survived out of 20 germlings isolated from each set. Other inter-reference-collection pairing sets such as IBUG $7_1$:20 X 7839:2, and IBUG $7_1$:7 X 7839:6 had lower survival rates, 14 and 16 SBIs respectively. The potentially reduced survival rate between 7444 and other collections could only be stated tentatively because of the low sample size. It was difficult to discern whether this truly indicated reduced $F_1$ hybrid vigor or fitness between collections, or was simply variation resulting from the harvesting technique.

Several previous workers have examined intercompatibility in this species complex. Neda *et al.* (1988) found *P. salmoneostramineus* to be compatible with *P. djamor*. Petersen (1992) and Petersen and Hughes (1993) reported that *P. ostreatoroseus* (Mexican collection) was compatible with *P. salmoneostramineus* (Japan). However, they also reported that two collections of the white to tan color classes, 3467 from Puerto Rico and 4326 from Mexico, were incompatible with *P. ostreatoroseus* and *P. salmoneostramineus* (Petersen and Hughes, 1993). In our study, both collections (3467 was a reference collection) were compatible with different *P. djamor* morphotypes from widespread geographic areas.

Petersen (1995) reported compatibility among pink, gray and white to tan morphological variants of this complex. Guzman *et al.* (1995) described two pink varieties of *P. djamor*, var. *roseus* and var. *salmoneostramineus*, as compatible with typical white *P. djamor*. In addition they mentioned that there were no micromorphological characters that distinguished these varieties. Studies by Cedano *et al.* (1993) and Guzman *et al.* (1995) also stated that basidiomata of these “pink” varieties or “species” could lose their color, making them indistinguishable from white-tan *P. djamor*. Interestingly, Cedano *et al.* (1993) reported that under intense light conditions the pink color was lost while Guzman *et al.* (1995) stated that the pink color was lost under low light conditions. Our study found that loss or retention of pink coloration was not correlated with light intensity.

**Species concepts and this study.** - Biological species concept. -- The widespread intercompatibility and interfertility reported for members of the *P. djamor* complex placed many geographically diverse collections into one biological species.

Phenetic species concept. -- Following the phenetic species concept there was no morphological dissimilarity that clearly subdivided the complex. There was a suggestion that the New Zealand collections examined were more similar to each other than to other collections of *P. djamor* but with much phenetic overlap.

Morphological species concept. -- Following a more traditional morphological species concept one could propose that the New Zealand collections represented an infraspecific unit within *P. djamor*, based on their unique characters of dark brown primordium coloration and lack of hygrophanous areas on the pileus, but certainly more collections are required to substantiate the consistency of these differences.

Phylogenetic species concept. -- "*Pleurotus djamor*" has been included in generic phylogenetic reconstructions using DNA sequences and isozymes (Vilgalys and Sun, 1994; Zervakis and Labarere, 1992; Zervakis *et al.*, 1994), and found to segregate independently from other taxa. On adjacent clades appear other taxa with dimitic hyphal construction. Phylogenetic relationships within the *P. djamor* complex have not been examined using these techniques. A study of *Pleurotus ostreatus* (Matsumoto and Fukumasa-Nakai, 1995) using restriction fragment length polymorphisms revealed that geographic distances between natural populations from Japan, Germany, Czechoslovakia, Hungary, France, Korea and U.S.A., were correlated with genetic divergence. Based on their results for *P. ostreatus* and the paucity of information on long-distance spore dispersal in mushrooms, genetic analyses such as DNA sequencing, RFLPs or protein electrophoresis appear to be the next major step in understanding gene flow among individuals and populations in the *P. djamor* hybrid swarm.

Pluralistic species concept. -- The three species concepts utilized in our study (biological, phenetic and morphological) provided similar results, with none furnishing consistent differences on which to base nomenclatural rank for any collections of *P. djamor* examined in this study. Several lines of evidence, however, suggest that there may be some differentiation among collections belonging to this complex. There was clustering of collections from the same broad geographic area in phenograms, albeit with collections from other areas interspersed. The micromorphological basidiome differences for New Zealand collections and the possible reduction in spore viability of hybrids from New Zealand reference collection 7444 also suggest some possible differentiation by geography. In addition, Petersen and Ridley (1996) found a New Zealand *Pleurotus* with multiple-species sexual compatibility, highlighting the need for caution in extrapolating species status based solely on intercompatibility between allopatric populations.

## APPENDIX

As a result of notes on field-collected and fruited basidiomata, intercompatibility tests to limit the circumscription, and phenetic analyses of micromorphological structures, the following morphological description of *Pleurotus djamor* is furnished:

Macromorphology. -- Pileus flabelliform, spathulate, petaloid, concave, reniform to infundibulliform; surface smooth, fibrillose to tomentose over stipe area; margin opaque, to hygrophanous, striate when moist, even when young becoming undulate-lobed to crenate in age, white, yellowish white (4A2,3), yellowish gray (4B2), grayish yellow (4B3), light yellow (4A4), orange-gray (5B2), grayish orange (5B3-5, 6B3, 4), yellowish brown (5D-F4), grayish brown (5D-F3, 6D, E3, 7D-F3), brownish orange (5C3, 4, 6C3-5, 7C4), orange-white (5-6A2), pale orange (5-6A3), brownish gray (4C2), orange-gray (5-6B2), brownish gray (5C2), brown (6D4-5, 6-7E4), dark brown (6F4-6, 7F4), yellowish brown (5E-F4), grayish red (7B3, 7B5, 8B3, 8C3-4), pinkish (7-8A2, 11A2), pale red (10A3), pink (11A3), light brown (6D4), brownish orange (6C3, 6C5), grayish orange (5B3, 6B4), pastel red (7A4), topinkish red (7-11A2). Lamellae decurrent to subdecurrent, unequal, occasionally anastomosing at base, thin, broad becoming narrower at margin, entire, white to yellowish-white (4A2-3), light yellow (4A4), yellowish gray (4B2), orange white (5-6A2), pale orange (6A3), grayish orange (5B6), pinkish white (7-11A2), pale red (7-10A3), pastel red(8A4), or grayish red(7B3). Stipe absent or short, 5-20 mm long, eccentric or lateral, equal to tapering downward, smooth, pubescent to tomentose, usually white to yellowish white (4A2-3), occasionally orange white (5A2), pinkish white (8-10A2), pale red (8A3), to grayish red (9B3). Spore print yellowish white (4A2-3), yellowish gray (3-4B2), grayish yellow (4B3), grayish beige (4C2), orange gray (5B2), grayish orange (5B3), pale orange(6A3), orange white (5-6A2), pinkish white (8-9), pale red (8A3), reddish gray (9B3), to grayish red (8B3). Lamella, stipe and spore color consistent with, but paler than pileus color (i.e. pale pink spore print always occurring with a pink pileus).

Micromorphology: Pileipellis: a thin epicutis of generative hyphae; hyphae slender to somewhat inflated, 3.4-8.3 μm diam, thin- to somewhat thick-walled (wall 0.4-1.1 μm thick), occasionally branched, clamped, repent to suberect, often forming fascicles. Pileus context monomitic when young, becoming dimitic with age; generative hyphae branched, septate with prominent clamp connections, 3.6-13.7 μm diam, thin- to thick-walled (wall 0.4-1.1 μm thick); skeletal hyphae 2.9-6.8 μm diam, thick-walled (wall 1.1-2.9 μm, creating a narrow lumen), not septate, rarely branched, often twisted, terminally attenuate. Hymenophoral trama: monomitic at first soon becoming dimitic, irregular; skeletal hyphae (2.8-6.8 μm diam, wall 0.7-2.5 μm thick), non-septate, unbranched; generative hyphae 4.0-10.8 μm diam, septate, branched,, thin-walled (wall 0.4-1.1 μm thick). Subhymenium: pseudoparenchymatous; cells 3.6-16.2(-28.8) μm diam, with interwoven hyphae 5.0-8.3 μm diam, thick-walled (wall 1.1-2.9 μm thick), Pleurocystidia 14.4-24.1 X 4.7-6.5 μm, abundant in notch of gill when present, subventricose-capitate to subventricose-mucronate, thin-walled, hyaline. Hyphae extended through the hymenium as pseudocystidia subventricose, cylindro-clavate to cylindric, 24.12-104.4 μm long, occasionally branched. Basidia: 16.2-32.0 X 4.7-7.9 μm, clavate, four-sterigmate, hyaline, thin-walled, clamped. Cheilocystidia: 14.0-35.3 X 4.7-11.9 μm, crowded on sterile lamella edge, hyaline, thin-walled, clavate, cylindric, subcylindric, ventricose, ventricose-rostrate (occasionally with long rostrum), subventricose, subventricose-capitate (occasionally bicapitate), subventricose-mucronate, subsphaeropendunculate, rarely with an apical halo drop.

Basidiospores: 6.8-13.3 X 3.6-5.8 μm, hyaline, smooth, thin-walled, inamyloid, cylindric, oblong, with distinct hilar appendix.

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# LEUCOAGARICUS VIRIDIFLAVOIDES, A NEW SPECIES FROM FLORIDA, WITH NOTES ON RELATED TAXA

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## ABSTRACT

A rare species of *Lepiota* s.l. with yellow flesh that bruises bluish-green was collected several times from a site in north central Florida in 1997. It is described here as a new species, *Leucoagaricus viridiflavoides*, related to *Lepiota viridiflava* Petch from Sri Lanka, *Leucoagaricus* "*cyanotinctus*" (TJB 7970) from Puerto Rico, and *Leucocoprinus sulphurellus* Pegler from the Lesser Antilles. *Leucocoprinus sulphurellus* is transferred to *Leucoagaricus* here, based on microscopic study.

## INTRODUCTION

The earliest accounts of *Lepiota* (Pers.) Gray s.l. in Florida are those of W.A. Murrill, who described 44 species as new during the time he was stationed at the University of Florida in Gainesville, Alachua County (1939a, 1939b, 1940, 1941a, 1941b, 1942, 1943, 1944, 1945, 1946, 1949,

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1951). The type localities for most of these species are in Alachua County, making it one of the most intensively studied and historically important regions in the state for this group of fungi, as well as for others. In addition to the species he described as new, Murrill (1949, 1972) cited nine other taxa from Florida widely recognized as lepiotaceous, for a total of 53 species. Singer (1944) noted the occurrence of *Smithiomyces mexicana* (Murrill) Singer in Florida, and recognized it as closely related to *Lepiota* s.l. More recently, field research and studies of more than 300 herbarium collections from Florida indicated the presence there of about 70 species in the family Lepiotaceae Roze (Akers 1997).

In 1997, a species of *Lepiota* s.l. previously unrecorded from Florida was collected several times at a site near Gainesville. Among the lepiotaceous taxa from Florida, this species was distinguished by having yellow flesh and a distinctive, bluish-green bruising reaction upon handling. These characteristics, along with micromorphological features, indicated similarities to *Leucocoprinus sulphurellus* Pegler from the Lesser Antilles (Pegler 1983), and *Lepiota viridiflava* Petch (Petch 1917) from Sri Lanka. Based on comparative study of available collections of lepiotaceous taxa with yellow flesh that bruises blue to bluish-green, including the type and other collections of *Leucocoprinus sulphurellus*, this unusual find from Florida was determined as a new species. It is presented here, along with a discussion of its affinities with other, comparable species.

## MATERIALS AND METHODS

Collections were obtained in the field and photographed *in situ*, to show habit and habitat. Notes were taken on macromorphological features, and specimens were deposited in the University of Florida mycological herbarium (FLAS). The holotype and other authentic collections of *Leucocoprinus sulphurellus* were borrowed from the mycological herbarium of the Royal Botanical Gardens, Kew (RBG, Kew). Another collection tentatively identified as *L. sulphurellus* was borrowed from the State University of New York—College at Cortland (CORT). Authors of fungal names are abbreviated according to Kirk and Ansell (1992), and abbreviations of Latin terms follow Stearn (1992).

Sections were cut by hand and mounted in 3% KOH and Melzer's reagent, following standard mycological methods (Smith 1949; Largent et al. 1977; Singer 1986). Basidiospores were mounted also in cresyl blue to test for metachromasy (Singer 1986). Optical microscopy studies were carried out at 400 X and 1000 X, and line drawings of microscopic features were made using a Leitz drawing tube. Size ranges were determined for basidia, basidiospores, cheilocystidia, pleurocystidia and elements of the pileipellis, based on ocular micrometer measurements of at least 12-15 elements of each type. Wall thickness of the basidiospores is shown in only one example from those illustrated. Colors noted in descriptions are personal interpretations.

## RESULTS

*Leucoagaricus viridiflavoides* Akers & Angels, sp. nov. Figs. 1 and 2

Species haec a *Leucocoprino sulphurello* praesertim differt pileo sordide roseo-brunneo, fuligino-brunneo, violaceo-brunneo, viridi-brunneo vel atro-griseo (non sulphureo) et sporis in cumulo pallide flavis (non albidis), 5.3-11(-13) x 3.4-5.1(-6) µm (non 6-7 x 3.7-4.5 µm), ellipsoideis ad arachiformibus (non ellipsoideis ad amygdaliformibus).
HOLOTYPUS. FLAS F56754.

Macromorphological features. **Pileus** 1.6-3.5 cm broad, convex to nearly plane, obtusely umbonate, margin nonstriate to occasionally minutely striate, often splitting radially almost to the disc, occasionally appendiculate, flesh moderately thick and firm; cuticle almost smooth to minutely appressed fibrillose-granulose, subviscid when moist, dull pinkish brown to dull sooty brown to dull violaceous brown to greenish brown to gray to almost black, intact at the disc, elsewhere thinning and becoming paler with expansion of the pileus, or rarely disrupting concentrically into squamules, exposing sulfur yellow context beneath, cuticle and context alike turning deep bluish-green upon handling, and drying a greenish-gray or yellowish-greenish gray. **Lamellae** free, broad, close to subdistant, pale sulfur yellow, bruising deep bluish-green where touched, trama turning pale dull pink when cut, drying yellow with blue-green-gray to yellow-green-gray edges, lamellulae present, few. **Stipe** 1.8-4.5 cm long, equal to subequal, up to 3 mm wide, sulfur yellow turning deep bluish-green where handled, glabrous, hollow, base occasionally minutely granulose-scaly in young specimens; partial veil present, superior, membranous, sulfur yellow, leaving marginal remnants and/or poorly formed annulus, occasionally evanescent. Odor mild, pleasant. Taste not sampled. **Spore print** pale yellow.

Micromorphological features. **Basidiospores** 5.3-11(-13) x 3.4-5.1(-6) µm (Q = 1.81); ellipsoidal to ovoid to amygdaliform to arachiform ("peanut-shaped"), smooth, hilar appendix prominent, wall thick (0.5 µm), germ pore indistinct to conspicuous with apex sometimes truncate as viewed in Melzer's reagent; hyaline in KOH, weakly to moderately dextrinoid (medium rusty orange to paler with yellow perispore in Melzer's reagent), metachromatic (endosporium pale reddish violet in cresyl blue, pore tract not metachromatic). **Basidia** 14.2-22 x 5.5-8 µm; clavate, tetrasterigmate; basidioles 12-16 x 5-8.5 µm, clavate; all hymenial elements hyaline in KOH. **Cheilocystidia** 29-51 x 7-13.2 µm, abundant, clavate to slightly ventricose or more often ventricose-rostrate with a well-developed apical appendage; apical appendage subcapitate, cylindrical or more often submoniliform with a globose capitellum; walls smooth, thin; pale ripe olive in water, hyaline in KOH. **Pleurocystidia** 27.5-36 x 6.2-18 µm, scattered, narrowly to stoutly clavate with apical appendage papillate to articulated, short-rostrate; walls smooth, thin; hyaline in KOH. **Pileipellis** of filamentous hyphae, occasionally branching and interweaving, septate to articulated, mostly recumbent but with some

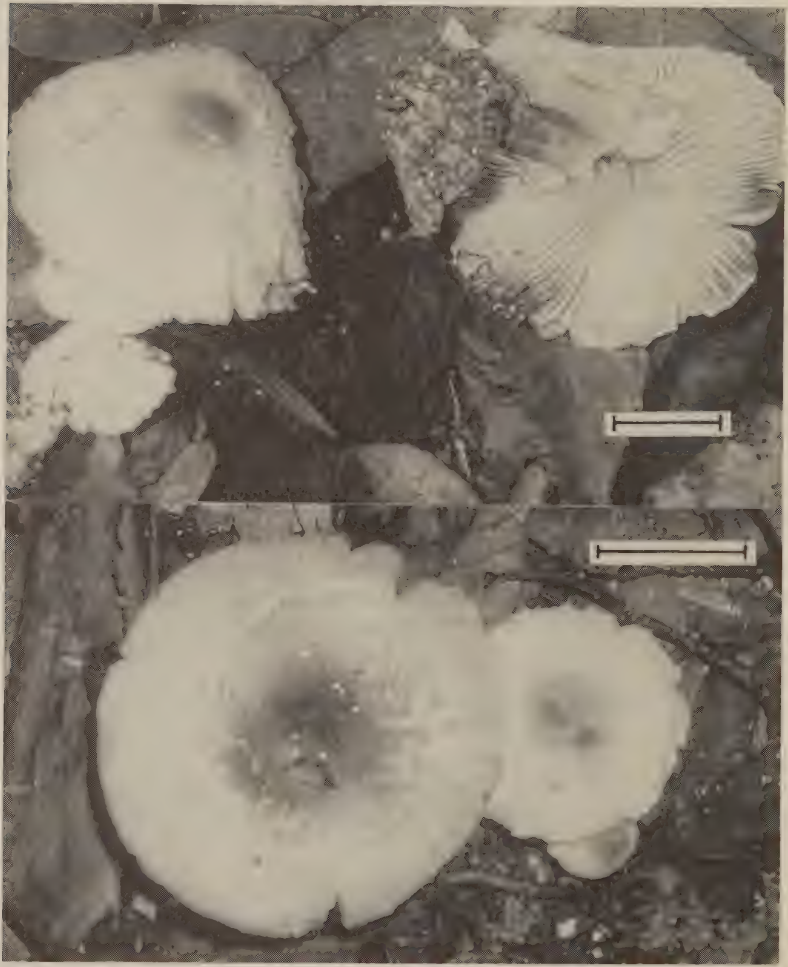

Fig. 1. Habit of *Leucoagaricus viridiflavoides* Akers & Angels. Note annulus and bruising reaction on stipe and lamellae (above, right). Scale bars = 1 cm.

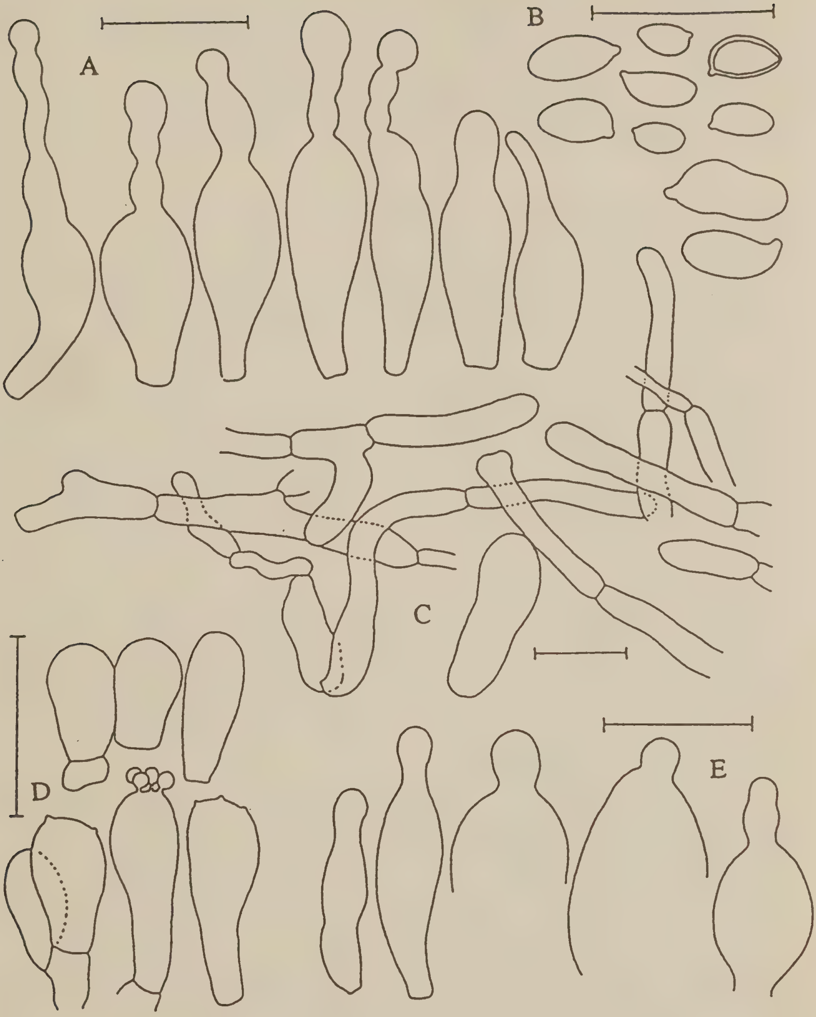

Fig. 2. Micromorphology of *Leucoagaricus viridiflavoides* Akers & Angels. A. Cheilocystidia. B. Basidiospores. C. Pileipellis. D. Basidia and basidioles. E. Pleurocystidia. All illustrations FLAS F56755. Scale bars = 20 µm.

short, variably ascending branches; elements 18-55 x 2.5-13 μm, subcylindric or "sausage-shaped" to slightly ventricose, terminal elements not differentiated; walls smooth, hyaline in KOH, slightly yellow in Melzer's reagent; vacuolar pigment pale yellow brown in KOH, with dark olive granules or vesicles present as viewed in Melzer's reagent; oleiferous hyphae present in trama below pileipellis, with content hyaline in KOH, golden in Melzer's reagent. **Clamp connections** absent.

Collections studied. USA, FLORIDA. Alachua County, San Felasco Hammock State Preserve, 4.5 miles WNW of Gainesville, terrestrial, scattered in mesic hammock of old sinkhole containing water elms (*Planera aquatica*); 23 July 1997, *S. Angels*, coll., *B. Akers*, det., FLAS 56754 (holotype); 16 July 1997, *S. Angels* and *J. Kimbrough*, coll., *B. Akers*, det., FLAS F56524; 3 Aug. 1997, *S. Angels*, *J. Kimbrough* and *B. Akers*, coll., *B. Akers*, det., FLAS F56755.

Additional collections studied. *Leucocoprinus sulphurellus*: MARTINIQUE. Regale, in banana plantation; 5 Aug. 1975, *J.P. Fiard*, coll., *D.N. Pegler*, det., KEW (M)59509 (holotype, Fiard 175). GUADELUPE. Basse Terre, Ste-Rose, Trace de Sofaia, solitary in mesophytic forest, 25 Oct. 1977, *D.N. Pegler*, coll. and det., KEW Pegler 3125. PUERTO RICO. Caribbean National Forest, El Yunque, Caimitillo Trail, approx. 650 m elev., N18 deg., 18', 9", W65 deg., 47', 11" (WGS84 map datum); solitary on soil, 29 June 1996, *T.J. Baroni*, coll. and det., CORT TJB 7970.

Comments. This is a rare species, known only from the locality cited above. The occurrence of water elms at the collection site suggests the possibility of some ecological association between them and this species, perhaps a preference for the leaf litter they produce as a saprobic mycelial substrate. However, a search for specimens at a few other local sites where water elms occur has so far proven negative, so this association has not been supported by further data.

The color scheme and stature of this species can cause confusion with species in the family Strophariaceae Singer & A.H. Sm. if the differing gill attachment is not noted. Furthermore, some of the species in that family, especially those containing psilocybin and psilocin (e.g., some species of *Psilocybe* [Fr.] P. Kumm.), can exhibit a striking blue bruising response to handling. When a yellow pigment is present in the flesh, this bruising can appear bluish-green, exactly like the bruising reaction observed in this species. Also contributing to the possibility of misidentification is the fact that the bluish-green bruising color can actually exude from the fresh specimens, affecting the spore print. Although droplets have not been observed, the bluish-green exudate can be rather copious, sometimes staining the fingers and even other surfaces touched afterwards. When the spore print was first taken, contact between the paper it was taken upon and the lamellae caused the paper to be stained bluish-green, which yielded a misleading impression of the spore color. After this was discovered, a less deceptive spore print was obtained by placing the pileus upon a simple mesh screen, thus keeping the lamellae out of contact with the paper while the spores were deposited upon it. The chemical nature of this bluish-green bruising and exudation is unknown. No data are available on the toxicity or edibility of this species.

Out of several pilei set up overnight for developing spore prints, one became infested with *Syzygites megalocarpus* Ehrenb.:Fr. This poses an addition to the list of fungi which have been reported as hosts of this mycoparasitic zygomycete (Kovacs and Sundberg 1994).

## DISCUSSION

Although not common in *Lepiota* s.l., blue to green bruising reactions have been noted in several species. For example, *Lepiota cyanescens* Beeli from Africa and *Lepiota viriditincta* (Berk. & Broome) Sacc. from Sri Lanka reportedly display such reactions with handling (Beeli 1927; Pegler 1972, 1986). However, these two species have white flesh rather than yellow, clearly setting them apart from the species studied here. *Lepiota caerulescens* Peck (= *Lepiota virescens* Morgan) from the USA likewise lacks yellow pigmented flesh. This species reportedly displays a combination of blue, green, and red tints upon handling (Morgan 1906), and dries with a bluish tint which does not persist very long in herbarium collections.

Apart from the taxa studied here, literature review indicated only one other taxon, *Lepiota sulphureocyanescens* Franco-Mol. from Colombia, with the unusual combination of yellow flesh and blue bruising. However, this species has basally truncate, spurred basidiospores which lack an apical pore. In addition, it lacks pleurocystidia, and has clamp connections, plus types of cheilocystidia and pileipellis which differ from the species from Florida described here (Franco 1994).

Based on literature review, the Florida collections compared closely with only two known species: *Lepiota viridiflava* Petch from Sri Lanka, and *Leucocoprinus sulphurellus* Pegler from the Lesser Antilles (Petch 1917; Pegler 1983). The features of these two species as reported in the literature compared so closely that questions were raised of how they were related, not only to the Florida taxon, but to each other as well. These similarities indicated a need for further study in order to clarify the affinities of these taxa.

Determining the disposition of *Lepiota viridiflava* collections proved problematic, because none are cited in the original description. Petch (1917) stated that it was collected in Peradeniya several times from flower beds between July and November, 1909. But unlike other agaric species described in the same article, there is no citation of a holotype or any other representative collections. Inquiries with the herbaria at Kew and Perideniya yielded no information to clarify this situation. Pegler (1986) included this species in his treatment of the agarics of Sri Lanka "for the sake of completeness" but also stated that no collections of it had been traced. By itself, the lack of any cited collections in Petch's description might be construed as a mere editorial oversight. But the failure of efforts to locate collections, when added to this fact, suggests there may be no extant holotype. The original diagnosis by Petch (1917) was the only available basis for comparisons with this species.

Comparative data on *Leucocoprinus sulphurellus* were obtained by study of the holotype and paratypes. One finding was that one of the paratypes, Pegler 3261 from St. Joseph, Dominica, was a small, non-lepiotaceous species, apparently misidentified. This collection is the only one reported from Dominica, so this island should be excluded from the known distribution for this species. A collection more recently obtained from Puerto Rico by Dr. Tim Baroni and tentatively identified as *L. sulphurellus*, TJB 7970, was also studied, with interesting results. A color photograph of this collection in its fresh state can be seen at the web site <www.cortland.edu/nsf/ga.html>.

Among the collections studied, the most telling comparisons pertained to the basidiospores. Although all collections had ellipsoidal, porate, metachromatic spores, significant differences were noted in terms of dimensions, length/width ratio, shape, aspect of germ pore, and response to Melzer's reagent. Based on these comparisons, TJB 7970 could not be confirmed as *Leucocoprinus sulphurellus*, and appears to represent a related, undescribed species, provisionally designated here as *Leucoagaricus "cyanotinctus"*.

The collections from Florida had a maximum basidiospore length greater than that of any of the other species studied here. Petch (1917) reported the basidiospore dimensions of *Lepiota viridiflava* as 5-8 x 3-4 µm. Pegler reported the basidiospores of *Leucocoprinus sulphurellus* as 6-7 x 3.7-4.5 µm, with a length/width ratio (Q) of 1.55, suggesting a similar length but slightly broader proportion than *Lepiota viridiflava*. A greater difference was noted, however, after study of Pegler's holotype (Fiard 175), which showed basidiospore dimensions of 5.1-6.5 x 3.8-4.3 µm. This indicated a length range and maximum length for basidiospores of *Leucocoprinus sulphurellus* lower than was originally reported by Pegler (1983). Q was recalculated as 1.43. *Leucoagaricus "cyanotinctus"* displayed basidiospores 6.5-8.1 x 4.0-5.1 µm, with Q = 1.57, distinctly longer than those of *Leucocoprinus sulphurellus* and wider than those of *Lepiota viridiflava*. None of these data corresponded well with the Florida material, which had basidiospores up to 11(-13) µm in length, and Q = 1.81.

Petch (1917) described the basidiospores of *Lepiota viridiflava* as "broadly oval, somewhat tapering towards one end", but did not report on the presence or absence of an apical germ pore. Pegler (1983) described the basidiospores of *Leucocoprinus sulphurellus* as "ellipsoid to amygdaliform, mostly with a small germ-pore truncating the apex." Study of Pegler's collections indicated the basidiospores as ellipsoidal to ovoid to occasionally amygdaliform, and showed a small germ pore, less than 1 µm in diameter. Basidiospores of *Leucoagaricus "cyanotinctus"* were more regularly ellipsoidal to ovoid, similar in shape and proportion to those of *Leucocoprinus sulphurellus*. However, the germ pore was more conspicuous, with a pronounced apical truncation 1-1.5 µm across, unlike any of the other taxa studied here. By comparison, the germ pore of the Florida collections was less than 1 µm in diameter, as in *Leucocoprinus sulphurellus*, but the shape of the spores was far more variable than other collections studied, with larger examples varying to "peanut-shaped", a feature not observed in other collections.

Basidiospores of *Leucocoprinus sulphurellus* were reported as dextrinoid by Pegler (1983). Study revealed their response to Melzer's reagent to be somewhat weak, with the endosporium showing as yellow to pale rusty orange. This same type of weak dextrinoid response was noted in the collections from Florida. *Leucoagaricus "cyanotinctus"*, on the other hand, displayed a much stronger, classic dextrinoid response. The reaction of basidiospores of *Lepiota viridiflava* to Melzer's reagent has not been reported.

In summary, basidiospores of *Leucocoprinus sulphurellus* were shorter and displayed little variation in shape compared with those of *Leucoagaricus viridiflavoides*, but both shared the features of a weak dextrinoid response and small germ pore. *Leucoagaricus "cyanotinctus"* had regularly ellipsoidal to ovoid basidiospores, similar in shape to those of *Leucocoprinus sulphurellus* but of greater length, plus a larger germ pore and strong dextrinoid response. This Puerto Rican collection consisted of a single basidiome with pileus 11 mm in diameter and stipe 25 x 1 mm, a stature smaller than that of the other species examined here. The form of the pileipellis was consistent with the other species, but of somewhat finer gauge, with fibrils not exceeding 10 µm in diameter. Micromorphological study likewise indicated exceedingly thin lamellae, only 50 µm thick as viewed in cross section. These dimensions, if typical for this taxon, suggest a species much more gracile than the other three discussed here.

The taxa studied here appear to be closely related, and may belong to a natural group at a sectional level. The critical features they share in common are: yellow flesh which bruises blue to green; membranous partial veil which typically does not form a well developed annulus; fibrillose pileipellis which does not typically undergo disruption into conspicuous squamules upon expansion of the pileus; ellipsoidal to ovoid basidiospores with an apical germ pore; pleurocystidia; and lack of clamp connections. Many details about *Lepiota viridiflava* remain unclear, including whether it has pleurocystidia, whether its basidiospores have a germ pore, and how strong a response to Melzer's reagent they display. However, the chance that it is a synonym of *Leucocoprinus sulphurellus* would seem to be lowered in light of indications that collections from neighboring regions, much less remote than Sri Lanka, represent different species. Although the data are not sufficient to render such a conclusion decisive, the case that

*Lepiota viridiflava* is probably a separate species, rather than a synonym of *Leucocoprinus sulphurellus*, is strengthened.

Although Pegler (1983) described his species as a *Leucocoprinus* based on the porate spore and the form of the cheilocystidia, he noted the non-striate margin as an unusual feature for this genus. Although some authors have placed all *Lepiota* s.l. species with porate basidiospores in *Leucocoprinus*, most modern workers restrict this genus to species with a plicate-striate pileus margin and hymenium containing brachybasidioles (Bon 1981; Singer 1986). Pegler did not report brachybasidioles in *Leucocoprinus sulphurellus*, and recent study confirmed their absence from the hymenium. Together with the lack of a plicate-striate margin, this casts doubt upon the generic placement of this species. Accordingly, the new combination *Leucoagaricus sulphurellus* (Pegler) Akers (basionym: *Leucocoprinus sulphurellus* Pegler, Kew Bull. Add. Ser. 9: 420. 1983) is proposed here for this species (HOLOTYPE: KEW [M]59509 [Fiard 175]).

The species studied here clearly belong in the tribe Leucocoprineae Singer by virtue of their metachromatic, porate spores. Most of the genera in this tribe, including *Macrolepiota*, *Leucocoprinus*, and *Chlorophyllum*, have defining features which exclude these species. By default, the genus which can best accommodate them is *Leucoagaricus* (Locq.) Singer. Accordingly, the new species from Florida is placed therein. Recent molecular work (Johnson and Vilgalys 1998; Johnson 1999) has indicated some support for *Leucocoprinus* and *Macrolepiota*, but *Leucoagaricus* is not well supported and seems to function as a taxonomic waste-basket for Leucocoprinean species that do not fit well into other, better-defined genera. *Leucoagaricus* species thus apparently represent a heterogeneous assemblage of disparate groups not all closely related, rather than a natural group. The species from Florida here described as new is placed in *Leucoagaricus* with this understanding, and no definite relationship with other species assemblages in this genus is expressed or implied.

The discovery of this species in Florida might have been less surprising had it come from a region for which records of fungi are lacking or comparatively scarce. The fact of its occurrence in Alachua County, the most studied region of the state, underscores the need for further research on fungi of Florida, and also reflects how much more there is to learn about *Lepiota* s.l.

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# STUDIES ON SCHIZOPORA FLAVIPORA S.L., WITH SPECIAL EMPHASIS ON SPECIMENS FROM TAIWAN

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## ABSTRACT

Specimens of *Schizopora flavipora* s.l. collected in Taiwan during 1987-1999 have been studied. Many type specimens of this group have been examined. Three species were determined from Taiwan: *Hyphodontia flavipora*, *H. nongravis*, and *H. tropica*. *Hyphodontia nongravis* is separated from the other two species in having thicker, effused-reflexed basidiomata, and encrusted cystidia. *Hyphodontia tropica* has smaller spores and slightly shorter basidiospores than *H. flavipora*. Separation of these three species was supported by compatibility tests. *Hyphodontia flavipora* has a wide distribution from temperate to subtropical regions of the world. *Hyphodontia nongravis* is hitherto known from Sri Lanka, Yunnan (China), and Taiwan. *Hyphodontia tropica* is a common species of subtropical and tropical belts in lowland Taiwan. Cultural studies are provided for all three species. Reports of *H. apacheriensis*, *H. paradoxa*, and *H. radula* in Taiwan, were not confirmed in this study, following examination of the reported specimens. *Hyphodontia subiculoides* is proposed as a new combination.

**Key words:** Corticiaceae, cultural studies, *Hyphodontia, Schizopora*, taxonomy, Taiwan.

## INTRODUCTION

*Schizopora* Velen. is a member of the resupinate polypores with microscopic and ultrastructural features resembling the non-poroid corticioid genus *Hyphodontia* J. Erikss. Common microscopic features include the suburniform basidia, characteristic hyphal and cystidial forms, and similar spore morphology. In culture, both genera produce drepanocysts and malocysts. Ultrastructurally, members of this group have dolipores with continuous parenthesomes (Langer, 1994). "Skeletals" are present in many poroid species (*Schizopora*), but are totally absent in non-poroid species (*Hyphodontia*). The "skeletals" help to provide tramal strength for the formation of the poroid hymenial surface. *Echinoporia* Ryvarden also belongs to this group and is characterized by its pileate basidiomata producing numerous conidia from hydnoid processes on the pileus surface. Molecular evidence inferred from ribosomal DNA sequences indicates that *Hyphodontia* and *Schizopora* spp. form a single clade (Langer, 1998). Distinctions between poroid and non-poroid hymenial surfaces may not be useful in separating genera. For example, the genera *Sistotrema* Fr. and *Trechispora* P. Karst. accommodate both poroid and non-poroid members. Hjortstam (1987) suggested treating monomitic species of *Schizopora* in *Hyphodontia,* a view which was adopted by Wu (1990). However, the problem has not been settled, since there are difficulties in recognizing hyphal mitism in some *Schizopora* species. For example, Hallenberg (1983) considered *Schizopora radula* to be a monomitic species, though skeletal-like hyphae are present. Nevertheless, "skeletals" may arise independently among different taxonomic groups, as a means of strengthening basidiomata. These structures may even arise within a genus, e.g. *Trechispora,* containing mostly monomitic and a few dimitic species.

The foregoing evidence all suggests that *Schizopora*, *Hyphodontia*, and *Echinoporia* are congeneric. *Hyphodontia* contains a substantially larger number of species than *Schizopora* and *Echinoporia*. However, nomenclaturally *Schizopora* has priority over the other two names. Langer *et al*. (1996) proposed rejecting the names *Xylodon* (Pers.) Gray (the oldest name for *Hyphodontia*) and *Schizopora* in favour of *Hyphodontia*, to avoid the disadvantageous changes in nomenclature. Langer and Vesterholt (in Knudsen and Hansen, 1996) proposed new combinations for two species which have long been treated in *Schizopora*: *Hyphodontia paradoxa* (Schrad.:Fr.) E. Langer & Vesterholt and *H. radula* (Pers.:Fr.) E. Langer & Vesterholt. In this paper, the author accepts the proposition of Langer *et al*. (1996), and regards *Schizopora* as a synonym of *Hyphdontia*.

Species delimitation has not been easy for certain *Schizopora* species (Hallenberg, 1983; Niemelä, 1987; Ryvarden, 1991). For example, differences between *Schizopora radula* and *S. paradoxa* are minor, and the separation between them has only been proved from microscopic studies and compatibility tests (Hallenberg, 1983; Niemelä, 1987).

A survey of *Hyphodontia* from Taiwan was presented by Wu (1990). Fifteen species of *Hyphodontia* containing six new taxa were proposed in this survey, including a new poroid, monomitic species, *H. niemelaei* Sheng H. Wu. Specimens of *Schizopora* were not studied at the same time, chiefly for two reasons. First, *Schizopora* was long regarded as a polypore. Secondly, species delimitation was found by the author to be problematic. Most *Schizopora* specimens from Taiwan have similar characters, but different species may be present among them. Most collections seem to belong to *Schizopora flavipora* s.l. This species has been reported as widespread from temperate to tropical countries (Langer, 1994), with some synonyms. *Schizopora* species have been reported twice from Taiwan in recent years: Langer (1994) reported specimens of *S. paradoxa* and *S. radula*, while Chang (1995) reported *S. apacheriensis* (Gilb. & Canf.) Gilb. & Ryvarden (≡*Hyphodontia apacheriensis* (Gilb. & Canf.) Hjortstam & Ryvarden) and *S. flavipora*. Collections of four Taiwanese species have been examined in this study. Specimens previously recorded as *S. apacheriensis*, *S. paradoxa*, and *S. radula* were found to be *H. tropica*, a new species very common in lowland Taiwan.

The primary purpose of this study was to clarify species delimitations of Taiwanese *S. flavipora* s.l., based on morphology, cultural studies, and intercompatibility tests. Secondly, many type specimens of possible synonyms of *S. flavipora* and related species were examined, with the intention of understanding the distinctions between species and their correct names.

Three species of *S. flavipora* s.l. were determined from Taiwan: *Hyphodontia flavipora*, *H. nongravis*, and *H. tropica*. The first two are proposed as new combinations, and the last is proposed as a new species. *Hyphodontia flavipora* has a wide distribution from temperate to subtropical regions of the world. *Hyphodontia nongravis* is so far known only from Sri Lanka, Yunnan (China), and Taiwan.

## MATERIALS AND METHODS

Materials for this study were collected in Taiwan during 1987-1999. All specimens and cultures studied are deposited at the herbarium of the National Museum of Natural Science, ROC (TNM).

Descriptions of basidiomata were based on dried specimens. Free-hand thin sections of basidiomata were prepared for microscopic studies. For observations and measurements of microscopic characters, 5% KOH was used as a mounting medium to ensure rehydration. Melzer's reagent (IKI) was employed to detect amyloidity and dextrinoidity. Cotton blue (CB) was used as a mounting medium to determine cyanophily. All statistical analyses of basidiospores were based on measurements of 30 spores from spore-prints.

Cultural description and species code system are basically from Nobles (1965) with amendments by Boidin and Lanquetin (1983). Minor modifications to Nobles' code system have been presented by many mycologists. The Nobles' code detailed by Nakasone (1990) is comprehensive and adopted in this study. Following Nakasone (1990) the mycelia were grown on 1.5% MEA instead of 1.25% MEA. In this study, plates were inverted to avoid accumulation of water produced by the mycelia. Inverted plates permit formation of a hymenium oriented as in nature. Nuclear staining of mycelia was made with Giemsa according to Boidin (1958). DAPI (4'-6'-diamidino-2-phenylindole) was used at a concentration of 0.25 μg/ml as a fluorescent stain for nuclei of basidiospores. Terminology for nuclear behavior is based on Boidin and Lanquetin (1984). The methods of cultural studies and determination of sexuality have been detailed by Wu (1996).

## TAXONOMY

***Hyphodontia flavipora*** (Cooke) Sheng H. Wu, *comb. nov.* (Figs. 1A-B, 2)

Basionym: *Poria flavipora* Cooke, Grevillea 15: 25. 1886.
≡ *Schizopora flavipora* (Cooke) Ryvarden, Mycotaxon 23: 106. 1985.

= *Schizopora trichilae* (van der Byl ) Ryvarden, Fungiflora: 553. 1980.
≡ *Polyporus trichiliae* van der Byl, S. Afr. J. Sci. 18: 262. 1922.

= *Tyromyces hypolateritia* (Berk.) Ryvarden, Fungiflora 608. 1980.
≡ *Poria hypolateritia* Berk. ex Cooke, Grevillea 15: 24. 1886.

= *Schizopora carneo-lutea* (Rodway & Cleland) Ktl. & Pouzar, Ceska Mycol. 33: 21. 1979.
≡ *Poria carneo-lutea* Rodway & Cleland, Pap. Proc. Roy. Soc. Tasmania, S. 18. 1930.

= *Poria phellinoides* Pilát, Bull. Soc. Mycol. France 51: 383. 1936.
≡ *Xylodon versiporus* var. *phellinoides* (Pilát) Domanski, Acta Soc. Bot. Poloniae, Warszawa 33: 168. 1964.
≡ *Schizopora phellinoides* (Pilát) Domanski, Acta Soc. Bot. Poloniae, Warszawa 38: 255. 1969.

= *Poria pseudoobducens* [Pilát, Atlas hub evrop., Praha, 3: 462. 1942] ex Pilát, Sborn. Nár. Mus. Praha, B, 9: 107. 1953.
≡ *Xylodon versiporus* var. *pseudoobducens* (Pilát ex Pilát), Domanski, Acta Soc. Bot. Poloniae, Warszawa 33: 167. 1964.

Basidioma resupinate, effuse, adnate, membranaceous, usually fibrillose. Context up to ca. 250 μm thick. Hymenial surface cream, pinkish cream, or buff, poroid, cracked; margin concolorous or paler, fairly determinate, shortly filamentous. Pores angular, 3-6 per mm, soon becoming split; tube layer up to 1 mm deep; dissepiments 50-150 μm thick.

Hyphal system seemingly dimitic; generative hyphae with clamp-connections. Context with fairly loose texture; generative hyphae variously oriented, ± horizontal near substratum, colourless, 2-4 μm diam., with 0.5-1 μm thick walls, occasionally finely encrusted. Trama with dense texture; skeletal-like hyphae colourless or yellow, 3-5.5 μm diam., with 0.7-1.5 μm thick walls, dominant in central trama; generative hyphae similar to those of context. Generative hyphae of dissepiment surface with finely encrusted apices. Capitate cystidia numerous, abundant in context, trama, and hymenial layer, colourless, 15-40 μm long, with 5-10 μm diam. globose apices, thin-

or slightly thick-walled, encrusted with brownish yellow resinous material. Basidia suburniform, 12-20 x 4-5 $\mu$m, 4-sterigmate. Basidiospores ellipsoid, adaxially flattened, smooth, thin-walled, 4-5.1 x 2.9-3.7 $\mu$m, IKI–, CB–.

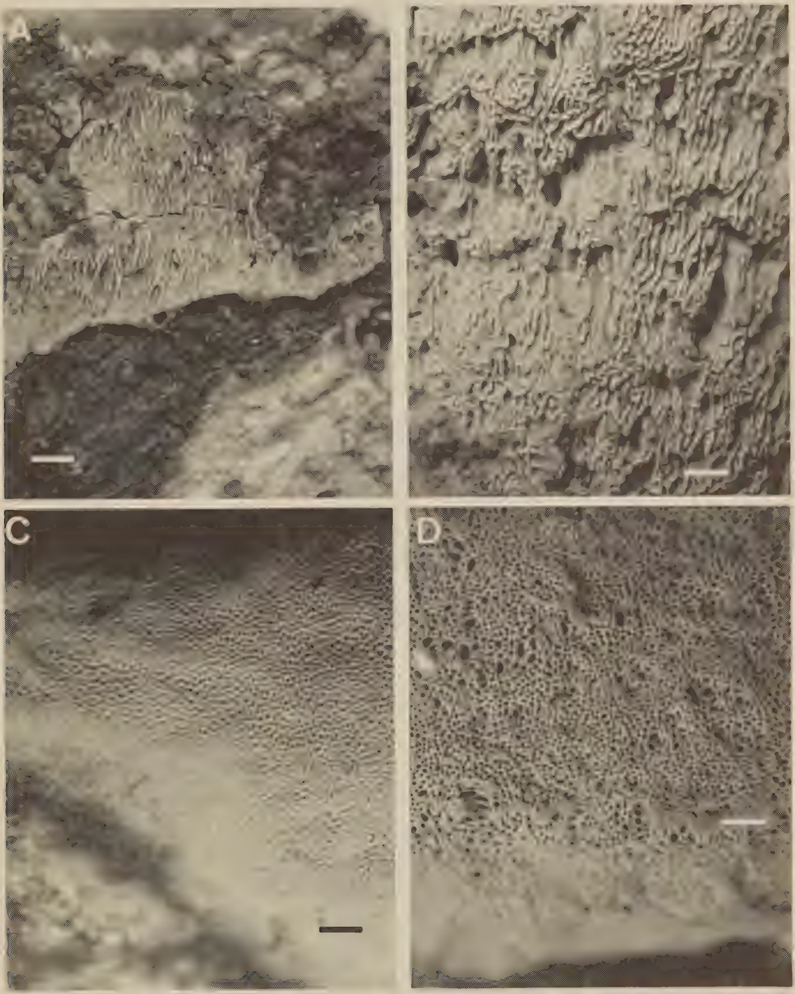

Fig. 1. Basidiomata. A. *Hyphodontia flavipora* (holotype). B. *H. flavipora* (*Wu 910724-8*). C. *H. tropica* (*Wu 910322*). D. *H. tropica* (*Wu 9211-106*). Scale bars = 1 mm.

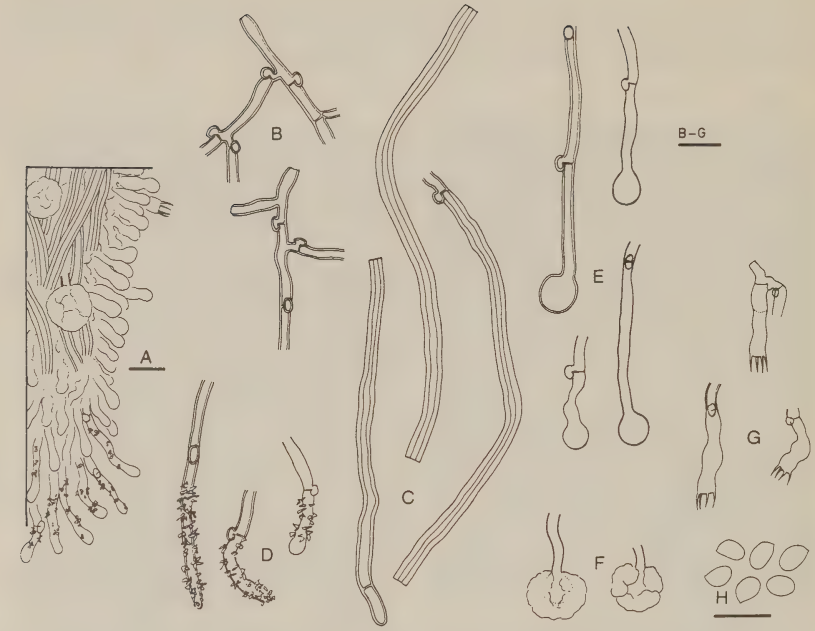

Fig. 2. *Hyphodontia flavipora* (*Wu 910724-8*). A. Part of vertical section of trama. B. Generative hyphae. C. Skeletal hyphae. D. encrusted hyphal tips. E. Capitate cystidia. F. Resinous material on the capitate cystidia. G. Basidia. H. Basidiospores. Scale bars = 10 $\mu$m.

*Specimens examined.* Taiwan. Taipei: National Taiwan University campus, on wood of gymnosperm, leg. S.H. Wu, 13-XI-1987, *Wu 871113* (TNM); 8-VII-1991, *Wu 910708-1* (TNM); on branch of *Melaleuca leucadendron*, leg. S.H. Wu, 3-VII-1989, *Wu 890703* (TNM). Neihu, on log for shiitake cultivation, 17-III-1988, *Wu 880317-1* (TNM). Yangmingshan, alt. 600 m, on branch of angiosperm, leg. S.H. Wu, 13-XII-1990, *Wu 901213-30* (TNM); 24-VII-1991, *Wu 910724-8* (TNM); 20-XI-1992, *Wu 9211-74*, *Wu 9211-75*, *Wu 9211-122*, *Wu 9211-145* (TNM); on bark of angiosperm, *Wu 9211-91* (TNM); on branch of *Pinus* sp., *Wu 9211-136* (TNM). Kungliao, alt. 200 m, on branch of angiosperm, leg. S.H. Wu, 25-XI-1990, *Wu 901125-7*, *Wu 901125-10* (TNM). Taoyuan: Shangpalin, alt. 1,500 m, on branch of angiosperm, leg. S.H. Wu, 3-VIII-1988, *Wu 880803-14* (TNM). Lalashan, alt. 1,600 m, on branch of angiosperm, leg. S.H. Wu, 4-VIII-1988, *Wu 880804-15* (TNM). Hsinchu: Chupu, on branch of angiosperm, leg. S.H. Wu, 16-II-1988, *Wu 880216-4* (TNM). Ilan: Jentse, alt. 400 m, on branch of angiosperm, leg. n.s., 28-II-1989, *Wu 890228-1* (TNM). Chilanshan, alt 400 m, on branch of angiosperm, leg. S.H. Wu, 18-XI-1992, *Wu 9211-2*, *Wu 9211-28, Wu 9211-53* (TNM); Fushan Botanical Garden, alt. 600 m, leg. W.N. Chou, 20-X-1993, *CWN 00016* (TNM). Taichung: Chiapaotai, alt. 850 m, on branch of angiosperm, leg. S.H. Wu & S.Z. Chen, 6-XII-1991, *Wu 911206-19* (TNM). Tungshi Forest Farm, alt. 300 m, on log of gymnosperm, leg. S.H. Wu, 13-V-1995, *Wu 9505-3* (TNM). Chingshan, alt. 2,000 m, on branch of *Pinus* sp., leg. T.T. Chang, IX-1992, *TFRI 448*. Anmashan, alt. 2,250 m, on branch of angiosperm, leg. S.H. Wu & H.J. Chan, 8-XI-1997, *Wu 9711-12* (TNM). Nantou: Shanlinhsi, alt. 1,700 m, on branch of angiosperm, leg. S.H. Wu, 8-VIII-1991, *Wu 910808-62* (TNM). Yushan National Park, Shenmu Forest Road, alt. 1,800 m, on branch of angiosperm, leg. S.H. Wu, 7-X-1992, *Wu 9210-81* (TNM). Piluhsi, alt. 2,100 m, on branch of angiosperm, leg. S.H. Wu, 4-XII-1991, *Wu 911204-37* (TNM); 14-IV-1993, *Wu 9304-54, Wu 9304-75* (TNM); alt. 2,000 m, on branch of angiosperm, leg. S.H. Wu, 14-IV-1993, *Wu 9304-108* (TNM). Neimaopu, Tachieshan, alt. 750 m, leg. S.H. Wu & J.Y. Tseng, 13-X.1994, *Wu 9410-57, Wu 9410-58* (TNM). Lienhuachih, alt. 700 m, on branch of angiosperm, leg. S.H. Wu, 5-VII-1993, *Wu 9307-28* (TNM); 8-X-1996, *Wu 9610-3* (TNM). Sun-Moon Lake, alt. 750 m, on branch of angiosperm, leg. S.H. Wu, 6-VII-1993, *Wu 9307-53* (TNM). Huisun Forest Farm, on branch of angiosperm, leg. S.H. Wu et al., 12-XII-1997, *Wu 9712-14* (TNM); alt. 700 m, on branch of *Cunninghamia lanceolata*, leg. S.H. Wu, 4-I-1999, *Wu 9901-23* (TNM). Hualien: Hsipao, alt. 750 m, on branch of *Coriaria intermedia*, leg. S.H. Wu & S.Z. Chen, 5-XII-1991, *Wu 911205-12, Wu 911205-18* (TNM). Chiayi: Yushan National Park, Nanhsi Forest Road, alt. 1 850 m, on branch of angiosperm, leg. S.H. Wu & S.Z. Chen, 13-X-1993, *Wu 9310-25* (TNM); alt. 1 950 m, on branch of angiosperm, leg. S.H. Wu & S.Z. Chen, 13-X-1993, *Wu 9310-54* (TNM); 3-V-1994, *Wu 9405-51* (TNM); alt. 1,800 m, on branch of angiosperm, leg. S.H. Wu & S.Z. Chen, 13-VI-1996, *Wu 9606-33* (TNM); alt. 2,000 m, on branch of angiosperm, leg. S.H. Wu & S.Z. Chen, 13-VI-1996, *Wu 9606-63* (TNM); alt. 1,950 m, on branch of angiosperm, leg. S.H. Wu & S.Z. Chen, 8-XII-1994, *Wu 9412-25, Wu 9412-36* (TNM). Pingtung: Kengting National Park, Lanjenshan, alt. 200 m, on branch of angiosperm, leg. S.H. Wu, 20-2-1992, *Wu 9202-19* (TNM); alt. 250 m, on branch of angiosperm, leg. S.H. Wu & S.Z. Chen, 17-VI-1992, *Wu 9206-59* (TNM). Russia. Siberia: Wasjuganje, ad corticem *Betulae verrucosae*, leg. Krawtzew, 30-VIII-1934 (holotype of *Poria phellinoides*, PRM 698242; pores ca. 6 per mm, spores 4-4.7 x 3-3.5 μm). Sri Lanka (holotype of *P. hypolateritia*, in Herb. Berkeley Nr. 1879, K; pores 6-8 per mm, spores 4-4.7 x 3-3.5 μm). Czechoslovakia. Carpatorossia: in silvis mixtis ad pratum Tiscora, prope Trebusany, on *Fagus silvatica*, leg. A. Pilát, VIII-1938 (holotype of *P. pseudoobducens*, PRM 494108; pores 6-7 per mm, spores 4-4.7 x 3-3.5 μm). South Africa, Durban (holotype of *Polyporus trichiliae*, Nr. 897, in K; pores 6-8 per mm, spores 4-4.8 x 3-3.6 μm). Venezuela (lectotype of *Poria flavipora*, in Herb. Berkeley Nr. 102, K; pores 6-7 per mm, spores 4-5 x 3-3.8 μm).

*Ecology*. Saprobic on branches and trunks of various kinds of angiosperms, rarely on gymnosperms.

*Distribution*. Pantropical to pantemperate.

*Statistical analyses of basidiospores*. *Wu 910724-8*: 4.1-5.1 x 2.9-3.3 µm (4.56 ± 0.25 x 3.06 ± 0.12 µm). *Wu 901125-10*: 4-4.7 x 3-3.3 µm (4.30 ± 0.23 x 3.11 ± 0.11 µm). *Wu 911204-37:* 4-5 x 2.9-3.4 µm (4.51 ± 0.30 x 3.13 x 0.15 µm). *Wu 911205-12*: 4-5 x 3-3.7 µm (4.48 ± 0.30 x 3.37 ± 0.21 µm). *Wu 9206-59*: 4-5 x 3-3.4 µm (4.45 ± 0.30 x 3.16 ± 0.14 µm). *Wu 9210-81*: 4-5 x 3-3.4 µm (4.41± 0.28 x 3.17 ± 0.13 µm). *Wu 9211-28*: 4.1-4.9 x 3-3.6 µm (4.53 ± 0.25 x 3.30 ± 0.15 µm). *Wu 9211-75*: 4-4.7 x 2.9-3.5 µm (4.26 ± 0.23 x 3.19 ± 0.15 µm). *Wu 9211-91*: 4-4.6 x 2.9-3.3 µm (4.25 ± 0.18 x 3.15 ± 0.10 µm). *Wu 9211-122*: 4-4.9 x 3.1-3.6 µm (4.46 ± 0.28 x 3.36 ± 0.14 µm).

*Compatibility tests*. Three haploid mycelia of *Wu 910724-8* were respectively paired with dikaryotic mycelia of *Wu 880216-4, Wu 901125-10, Wu 911204-37, Wu 911205-12, Wu 9211-145, Wu 9401-4*, and *Wu 9410-57*. Compatible results were obtained from all crossings. These monokaryons were found to be incompatible with dikaryotic mycelia of *Wu 9211-106* and *Wu 9211-153* (both are *Hyphodontia tropica*).

*Cultural description* (combined from polysporous mycelia of *Wu 910724-8* and *Wu 9410-57*). Colony radius respectively of 12-14 mm, 35-40 mm, and 60-65 mm for 1, 2, and 3 weeks of growth. Plates covered by 4 weeks of growth. Growth for 2 weeks: Advancing zone even. Mat white. Aerial mycelium almost absent. Advancing hyphae colourless, clamped, 2-5 µm diam., thin-walled. Growth for 6 weeks: Mat white. Aerial mycelium slightly pellicular to absent. Hyphal system monomitic. Hyphae colourless, clamped, sparsely or moderately ramified, 1.5-7 µm diam., thin-walled, occasionally swollen. Cystidia clavate, occasionally constricted in the middle, colourless, 15-30 x 6-10 µm, thin-walled. Crystals present (rod-like in *Wu 910724-8*). No distinct odour. Not fruiting.

*Oxidase reactions*. TAA: – , 0; – , 0. GAA: ++++, 0; ++++, tr. TYA: – , tr. to 15; – , 10-40.

*Species code*. 2a, 3, 13, 32, 36, 38, 44, 54, 60, 61.

*Sexuality*. Tetrapolar ($A_1B_1$: 1, 6, 7; $A_1B_2$: 3; $A_2B_1$: 2, 5, 9; $A_2B_2$: 4, 8). This study was based on *Wu 9410-57*.

*Nuclear behaviour*. Normal. Basidiospores uninucleate, monosporous mycelium uninucleate, polysporous mycelium dikaryotic (studied from *Wu 9410-57*).

*Remarks*. Information derived from this study and previous studies by other mycologists indicates that *H. flavipora* is a species widely distributed from temperate to tropical regions of the world. Examination of type specimens of possible synonyms has shown that most synonyms previously proposed are confirmed by this study. The synonyms are as listed above. *Poria carneo-lutea* has been regarded as a synonym of *Schizopora flavipora* (Niemelä, 1987; Langer, 1994). The type specimen of *P. carneo-lutea* is:

Australia. Bullahdelah, Aug. 1919, leg. Wakefield, no. 10. (HO). The author tried to borrow this type specimen for study and a specimen labelled as "part of type" was kindly sent by HO. This specimen is *HO 104194*, and according to the label its collection information is: New South Wales. Bulladelah, c. 1928, pro. collected by J.B. Cleland (Rodway Herb. 160). This specimen represents *H. flavipora*, bearing pores 5-6 per mm, and spores 4-5 x 3-4 μm.

***Hyphodontia nongravis*** (Lloyd) Sheng H. Wu, *comb. nov.* (Figs. 3A-C, 4)
Basionym: *Polyporus nongravis* Lloyd, Mycol. Writ. 6: 891. 1919.

Basidioma resupinate, effuse-reflexed, sometimes with slightly pileate margins, spongy or harder, ± adnate, membranaceous, fibrillose. Context up to ca. 2 mm thick in the reflexed part, much thinner in the effused part. Hymenial surface cream, poroid; margin paler, fairly determinate, shortly filamentous. Pores angular, 3-4 per mm; tube layer up to 2 mm deep, dissepiments 50-150 μm thick. Abhymenial surface of pileus cream, villose.

Hyphal system dimitic; generative hyphae clamped. Context with fairly loose texture; generative hyphae colourless, 1.5-3.5 μm diam., thin-walled; skeletal hyphae colourless, 2.5-5.5 μm diam., with 0.7-1.5 μm thick walls, with distinct lumen. Trama with moderately dense texture; generative hyphae and skeletal hyphae similar to those of context. Capitate cystidia numerous, distributed in context, trama, and hymenial layer, colourless, up to 100 μm long, with 6.5-12.5 μm diam. swollen apices, thin- or slightly thick-walled, encrusted with brownish yellow resinous material. Encrusted cystidia distributed in hymenium, colourless, cylindrical, 15-30 x 3-6 μm (encrustation excluded), usually thick-walled. Basidia suburniform, 12-18 x 4.5-5.5 μm, 4-sterigmate. Basidiospores ellipsoid, adaxially flattened, smooth, thin-walled, guttulate, 4-5.2 x 3.3-4 μm, IKI – , CB – .

*Specimens examined*. Sri Lanka. Peradeniya, leg. T. Petch (holotype, BPI). China. Yunnan: Yiliang, Shiaotsaopa, Houho, alt. 2,000 m, leg. S.H. Wu & J.Y. Tseng, 18-IX-1998, *Wu 9809-97* (TNM). Taiwan. Nantou: Huisun Forest Farm, alt. 600 m, on branch of *Schima superba*, leg. S.H. Lin, 1.VI.1997, *Wu 9706-1* (TNM). Pingtung. Kengting National Park, Lanjenshan, alt. 250 m, on branch of angiosperm, leg. S.H. Wu, 17-VI-1992, *Wu 9206-73* (TNM).

*Ecology*. Saprobic on branches of angiosperms.

*Distribution*. Sri Lanka, Yunnan (China), Taiwan.

*Compatibility tests.* Three haploid mycelia of *Wu 910724-8* (*Hyphodontia flavipora*) were respectively paired with dikaryotic mycelium of *Wu 9706-1* (*H. nongravis*). Incompatible results were obtained from all crossings.

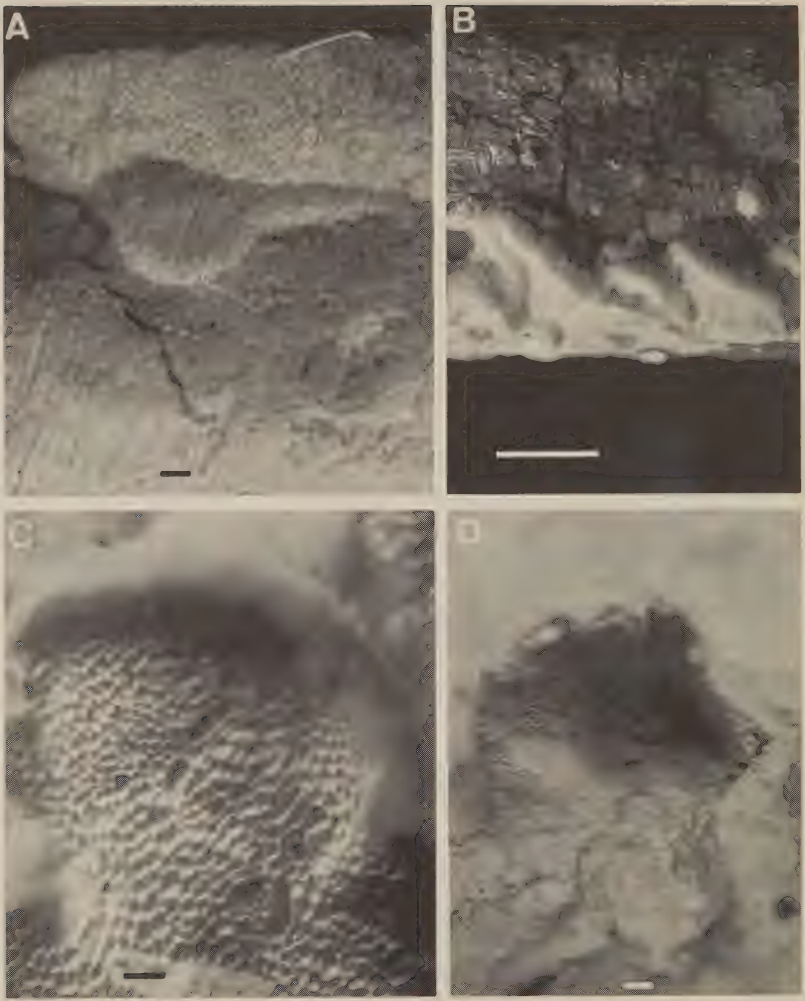

Fig. 3. Basidiomata. A. *Hyphodontia nongravis* (holotype). B. *H. nongravis* (*Wu 9706-1*). C. *H. nongravis* (*Wu 9706-1*). D. *H. subiculoides* (holotype). Scale bars = 1 mm.

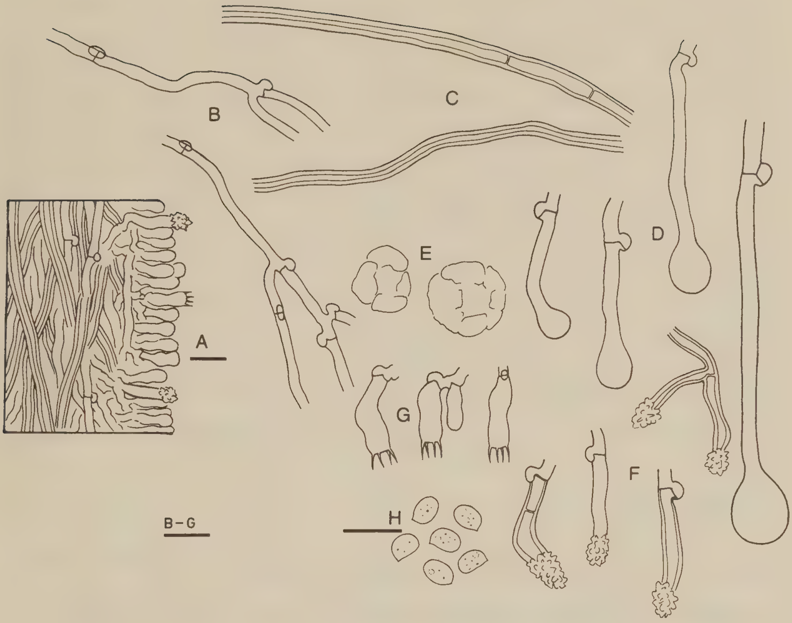

Fig. 4. *Hyphodontia nongravis* (*Wu 9706-1*). A. Part of vertical section of trama. B. Generative hyphae. C. Skeletal hyphae. D. Capitate cystidia. E. Resinous material on the capitate cystidia. F. Encrusted cystidia. G. Basidia. H. Basidiospores. Scale bars = 10 µm.

*Cultural description* (mycelium from woody substratum of *Wu 9706-1*). Colony radius respectively of 8-13 mm, 35-40 mm, and 62-67 mm for 1, 2, and 3 weeks of growth. Plates covered by 4 weeks of growth. Growth for 2 weeks: Advancing zone even. Mat white, fairly sparse. Aerial mycelium absent, ± zonate. Advancing hyphae colourless, clamped, 2.5-5 µm diam., thin-walled. 6 wk growth: Mat white. Aerial mycelium absent. Hyphal system monomitic. Hyphae colourless, clamped, moderately ramified, 2-6 µm diam., thin-walled, guttulate. Arthroconidia present, colorless, 7-13 x 3-5 µm, slightly thick-walled. Cubical crystals present. No distinct odour. Not fruiting.

*Oxidase reactions*. TAA: – , 0; – , 0; GAA: ++++, 0; ++++, 0; TYA: – , 0; – , 0.

*Species code*. 2a, 3, 13, 35, 36, 38, 44, 54.

*Remarks*. Ryvarden (1990) regarded *Polyporus nongravis* as a synonym of *Schizopora* *flavipora*. Macroscopically the former is separated from the latter in having much thicker basidiomata, which are usually reflexed towards margins. Microscopically, *H. nongravis* differs from *H. flavispora* in having encrusted cystidia. The "skeletals" of *H. flavipora* and *H. tropica* are usually confined to the tramal region, while these elements are distributed in the contextual and tramal regions of *H. nongravis*. Compatibility tests have been performed between *H. nongravis* and *H. flavipora* in this study, because of the similarity in their basidiospores. Negative results were obtained as noted above.

In possessing ± pileate basidiomata and encrusted cystidia, *Hyphodontia nongravis* resembles *Schizopora cystidiata* David & Rajchenb. It differs from the latter in having skeletal hyphae and capitate cystidia, additionally having slightly shorter basidiospores. The pileate basidiomata and encrusted cystidia are reminiscent of the genus *Echinoporia*. Two species of *Echinoporia* have a common feature, *i.e.*, hydnoid processes on the pileus surface from which numerous conidia are produced. Hydnoid processes and conidia have not been found from basidiomata of *H. nongravis*, but conidia were produced in culture (see above). *Hyphodontia nongravis* may represent an intermediate form between *H. cystidiata* and *Echinoporia*.

**Hyphodontia tropica** Sheng H. Wu, *sp. nov.* (Figs. 1C-D, 5)

Basidiocarpus effusus, membranaceus; superficies hymenialis poroidea. Systema hypharum dimiticum; hyphae fibulatae. Basidia suburniformia, 12-18 x 4-5 μm, 4 sterigmatibus. Basidiosporae late ellipsoideae, laeves, tenuitunicatae, 3.7-4.3 x 2.8-3.3 μm, IKI – , CB – .

Basidioma resupinate, effuse, adnate, membranaceous, usually fibrillose. Context up to 200 μm thick. Hymenial surface cream, pinkish cream, or buff, poroid, cracked when old; margin concolorous or paler, fairly determinate, shortly filamentous. Pores angular, 5-9 per mm; tube layer up to 200 μm deep; dissepiments 50-150 μm thick.

Hyphal system seemingly dimitic; generative hyphae clamped. Context with fairly loose texture; generative hyphae variously oriented, ± horizontal near substratum, colourless, 2-4 μm diam., with 0.5-1 μm thick walls, occasionally finely encrusted. Trama with dense texture; skeletal-like hyphae yellow or colourless, 2.5-5 μm diam., with 0.7-1.5 μm thick walls, dominant in central trama; generative hyphae similar to those of context. Generative hyphae of dissepiment surface with finely encrusted apices. Capitate cystidia distributed in context, trama, and hymenial layer, colourless, 20-40 μm long, with 5-10 μm diam. globose apices, thin- or slightly thick-walled, encrusted with brownish yellow resinous material. Basidia suburniform, 12-18 x 4-5 μm, 4-sterigmate. Basidiospores broadly ellipsoid, smooth, thin-walled, 3.7-4.3 x 2.8-3.3 μm, IKI – , CB – .

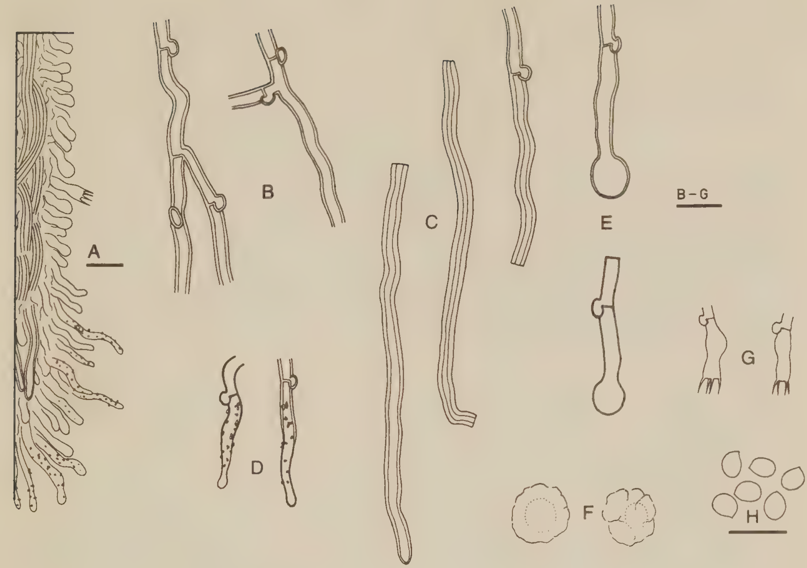

Fig. 5. *Hyphodontia tropica* (*Wu 910322*). A. Part of vertical section of trama. B. Generative hyphae. C. Skeletal hyphae. D. encrusted hyphal tips. E. Capitate cystidia. F. Resinous material on the capitate cystidia. G. Basidia. H. Basidiospores. Scale bars = 10 $\mu$m.

*Specimens examined.* Taiwan. Taipei: National Taiwan University campus, on branch of angiosperm, leg. S.H. Wu, 22-III-1991, *Wu 910322* (TNM). Yangmingshan, alt. 600 m, on bark of angiosperm, leg. S.H. Wu, 20-XI-1992, *Wu 9211-92* (TNM); on branch of angiosperm,_*Wu 9211-106, Wu 9211-123, Wu 9211-153* (TNM). Ilan: Chilanshan, alt. 400 m, on branch of angiosperm, leg. S.H. Wu, 18-XI-1992, *Wu 9211-5, Wu 9211-35, Wu 9211-36, Wu 9211-38, Wu 9211-41, Wu 9211-50* (TNM). Taichung: Chiapaotai, alt. 850 m, on branch of angiosperm, leg. S.H. Wu & S.Z. Chen, 6-XII-1991, *Wu 911206-29, Wu 911206-31, Wu 911206-43, Wu 911206-56* (TNM). Takeng, Walking Road No. 2, leg. S.Z. Chen, 7-XI-1993, *Wu 9311-5*. Tungshi Forest Farm, alt. 300 m, on branch of *Cryptomeria japonica*, leg. S.H. Wu, 13-V-1995, *Wu 9505-4* (TNM). Da-Ken, Kwan-Yin Mountain Area, trail to the Buddah-tempel, ca. 500 m, leg. E. & G. Langer, 15-VI-1990, *FO 41552* (originally identified by Langer (1994) as *Schizopora radula*) (pores 5-6 per mm, spores 3.8-4.2 x 3-3.3 $\mu$m). Nantou: Lienhuachih, alt. 700 m, on branch of angiosperm, leg. S.H. Wu, 1-V-1988, *Wu 880501-38* (TNM); 19-VI-1991, *Wu 910619-8* (TNM); 8-X-1996, *Wu 9610-7* (TNM); on trunk of angiosperm, leg. S.H. Wu, 13-VII-1997, *Wu 9707-9* (TNM); leg. E. & G. Langer, F. Oberwinkler, 9-VII-1990, *FO 42521* (originally identified by

Langer (1994) as *S. paradoxa*) (pores ca. 6 per mm, spores 3.9-4.3 x 2.9-3.2 μm). Huisun Forest Farm, alt. 770 m, on branch of angiosperm, leg. S.H. Wu et al., 12-XII-1997, *Wu 9707-13* (TNM). Kaohsiung: Liukuei, Shanping, alt. 750 m, on branch of gymnosperm, leg. S.H. Wu, 7-XI-1991, *Wu 911107-25* (TNM); on branch of angiosperm, *Wu 911107-30, Wu 911107-40, Wu 911107-42* (TNM); leg. S.H. Wu & S.Z. Chen, 21-XII-1993, *Wu 9312-6* (TNM); 22-XII-1993, *Wu 9312-11, Wu 9312-22, Wu 9312-57, Wu 9312-68* (TNM). Pingtung: Kengting National Park, Lanjenshan, alt. 150 m, on branch of angiosperm, leg. S.H. Wu, 17-VI-1992, *Wu 9206-62* (TNM); alt. 250 m, on branch of angiosperm, leg. S.H. Wu, 31-VIII-1994, *Wu 9408-32* (TNM). Kengting, on branch of angiosperm, leg. T.T. Chang, II-1992, *TFRI 244* (originally identified by Chang (1995) as *Schizopora apacheriensis*) (pores 7-9 per mm, spores 3.8-4.3 x 3-3.3 μm). Taitung: Orchid Island, between nuclear waste storage and Tasenshan, alt. 100 m, on branch of angiosperm, leg. S.H. Wu & J.Y. Tseng, 29-IV-1997, *Wu 9704-53, Wu 9704-71, Wu 9704-73* (TNM).

*Ecology.* Saprobic on branches and trunks of various kinds of angiosperms, rarely on gymnosperms.

*Distribution.* So far known only from lowland Taiwan.

*Statistical analyses of basidiospores. Wu 911206-31*: 3.7-4.2 x 2.8-3.1 μm (3.84± 0.15 x 2.91 ± 0.10 μm). *Wu 9211-36*: 3.9-4.3 x 2.9-3.2 μm (4.08 ± 0.12 x 3.06 ± 0.10 μm). *Wu 9211-92*: 3.8-4.3 x 2.9-3.3 μm (3.95 ± 0.13 x 3.06 ± 0.14 μm). *Wu 9211-106*: 3.8-4.3 x 2.9-3.3 μm (4.05 ± 0.14 x 3.09 ± 0.14 μm). *Wu 9211-153:* 3.8-4.2 x 3-3.3 μm (4.00 ± 0.13 x 3.14 ± 0.11 μm).

*Compatibility tests.* Three haploid mycelia of *Wu 9211-153* were respectively paired with dikaryotic mycelia of *Wu 910322* and *Wu 9211-106*. Compatible results were obtained from all crossings. These monokaryons were found to be incompatible with dikaryotic mycelia of *Wu 910724-8* and *Wu 911204-37* (both are *Hyphodontia flavipora*).

*Cultural description* (combined from polysporous mycelia of *Wu 9312-11* and *Wu 9211-153*). Colony radius respectively of 10-13 mm, 35-42 mm, and 65-73 mm for 1, 2, and 3 weeks of growth. Plates covered by 4 weeks of growth. Growth of 2 weeks: Advancing zone even. Mat white. Aerial mycelium absent to pellicular. Advancing hyphae colourless, clamped, 2.5-5.5 μm diam., thin-walled. Growth for 6 weeks: Mat white or slightly yellow. Aerial mycelium slightly pellicular. Hyphal system monomitic. Hyphae colourless, clamped, moderately ramified, 1.5-6.5 μm diam., thin-walled. Cystidia capitate or clavate, colourless, 12-25 x 6-12 μm, thin-walled. Crystals present. No distinct odour. Not fruiting.

*Oxidase reactions.* TAA: – , 0; – , 0. GAA: +++, 0; +++, tr. TYA: – , 10-30; – , 15-70.

*Species code*. 2a, 3, 13, 32, 36, 38, 44, 54, 60, 61.

*Sexuality*. Tetrapolar ($A_1B_1$: 1, 7; $A_1B_2$: 4; $A_2B_1$: 3, 5, 9; $A_2B_2$: 2, 6,8). This study was based on *Wu 9312-11*.

*Nuclear behaviour*. Normal. Basidiospores uninucleate, monosporous mycelium uninucleate, polysporous mycelium dikaryotic (studied from *Wu 9312-11*).

*Remarks*. *Hyphodontia tropica* is a common species in the subtropical and tropical belts of lowland Taiwan. All collections were made below 850 m. *Hyphodontia flavipora* is also a common species in Taiwan, but has a wider range of climate adaptation. It occurs in Taiwan from lowland to above 2,000 m. *Hyphodontia tropica* can easily be confused with *H. flavipora*, but some features separate them. The hymenial surface of *H. tropica* has comparatively smaller pores, which usually remain intact when old. However, the pores of *H. flavipora* tend to split soon. Microscopically, *H. tropica* has slightly shorter basidiospores. Careful measurement of spores can separate these two species. *Polystictus subiculoides* Lloyd has always been regarded as conspecific with *H. flavipora* (Ryvarden and Johansen, 1980; Langer, 1994), and the holotype (*C.G. Lloyd 48723,US0315674*, BPI) has been examined in this study. This specimen has pores ca. 6 per mm and spores 3.7-4.2 x 2.8-3.1 μm, which are similar to *H. tropica*. However, this type specimen has both resupinate and distinctly pileate parts, the hymenium restricted to the pileate part (Fig. 3D). This kind of macoscopic feature was not found among specimens of *H. flavipora* and *H. tropica*. Therefore, *P. subiculoides* appears to be an independent species. A new combination for this species is proposed as follows:

***Hyphodontia subiculoides*** (Lloyd) Sheng H. Wu, *comb. nov.*
Basionym: *Polystictus subiculoides* Lloyd, Mycol. Writings 7: 1331. 1927.

## ACKNOWLEDGMENTS

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# BATTARREA PHALLOIDES AND B. STEVENII, INSIGHT INTO A LONG-STANDING TAXONOMIC PUZZLE

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## Abstract

Two species of *Battarrea* Pers., *B. phalloides* and *B. stevenii,* have been cited in Europe. According to some authors, *B. phalloides* has a gelatinous volva and smaller basidiomata than *B. stevenii*. However, other authors consider that these characters are not enough to separate the taxa at the species level, and consider *B. stevenii* a synonym of *B. phalloides*. The main purpose of this work was to try to resolve the boundaries between the two taxa, not only based on macroscopic and microscopic characters, but also through molecular techniques. Thirty-five basidiomata from different geographical areas (Austria, Burundi, France, Hungary, Italy, Mexico, Spain) were examined to define the macroscopic and microscopic characters. The collections were investigated with regard to basidioma size, presence or absence of gelatinous hyphae in the volva, spore size and spore ornamentation. No differences were observed in spore size. From 16 basidiomata, amplifications of the ITS regions, including 5.8S rDNA were obtained. No gelatinous hyphae were found in the volva of the *B. phalloides* basidiomata, and no other morphological characters support the separation of the two taxa. The sequence alignment of the specimens has no unambiguous parts and the sequence dissimilarities within *B. phalloides* specimens are higher than those between *B. stevenii* and *B. phalloides*, suggesting that both taxa belong to the same species.

**Key words:** *Battarrea, Tulostomatales,* Basidiomycotina, taxonomy, rDNA.

## INTRODUCTION

Young specimens of the genus *Battarrea* Pers. (spelling according to HAWKSWORTH *et al.*, 1995[1]) show globose or pyriform basidiomata, while mature basidiomata of the genus are stalked with a campanulate peridium. *Battarrea phalloides* (Dickson) Pers. is the type-species of the genus. According to WATLING *et al.* (1995) the other European species, *B. stevenii* (Lib.) Fr., differs in the more orange tawny slightly larger (5-6.5 x 5.75-7 µm as opposed to 4.5-5.25 x 4.5-5.75 µm), less ornamentated basidiospores, much greater basidioma size, more coarsely scaly stipe and lack of mucilage in the volva and stipe medulla. The gelatinous volva seems to be the significant character that separates both taxa (MAUBLANC & MALENÇON, 1930; CUNNINGHAM, 1932; MORAVEC, 1958). REID (1985) confirms the presence of gelatinous tissues in material from the Channel Islands. However, HOLLOS (1904) was the first to clearly defend the idea of a single polymorphic species and gave a remarkable iconography (pl. II and IIbis) of specimens of all over the world. Later REA (1942) after studying 25 specimens from southern California concluded that *B. stevenii* may be considered a synonym of *B. phalloides*. MORENO *et al.* (1995) have identified collections from Mexico and Spain as *B. stevenii*, although they believe that *B. phalloides* ss. str. may possibly be the same.

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[1] The original spelling being *Batarrea*, the only admissible modification under Art. 60.1 is the one supported among others by HOLLOS (1904) and followed by many authors including HAWKSWORTH *et al.* (1995), to write *Battarrea*. The reason is that the person's name was Battarra. The spelling *Battarraea* is contrary to the code for this is not a correction of an orthographical or typogracial error but a standardization which is not covered by Art. 60.11 which does not apply to generic names.

The main purpose of this work was to address this unresolved question: Do *B. phalloides* and *B. stevenii* belong to one taxon or two? A morphological study of European collections of the genus *Battarrea*, using both macroscopic and microscopic characters was supplemented with analysis of the ITS regions of the rDNA.

## MATERIAL AND METHODS

*Material*.- Thirty-five basidiomata were examined to define the macroscopic and microscopic characters (Table 1).

*Morphological characters*.- The samples were prepared for SEM according to MARTÍN & ROCABRUNA (1988) and studied under a Hitachi S-2300 SEM. For the identification of the basidiomata, the criteria of WATLING *et al.* (1995) were followed.

*Molecular analysis*.- Total DNA was isolated following the protocols of GARDES *et al.* (1991) or WHITING *et al.* (1997) from 16 specimens referred in Table 1. Primer pairs ITS1F/4, ITS5/4 and ITS1/4 were used to obtain amplifications of both ITS regions, including the 5.8S of the ribosomal RNA gene cluster and small flaking parts of the SSU and LSU genes; primer pairs ITS1/2 and ITS3/4 were used to obtain separately amplificacions of ITS1 and ITS2 regions. Primers were described in WHITE *et al.* (1990) and GARDES & BRUNS (1993). Amplificacions were done using the standard procedure described in WHITE *et al.* (1990) or Ready-to-Go® PCR Beads (Amersham-Pharmacia Biotech) as mentioned in WINKA *et al.* (1998). Amplification products were cleaned using the QIAquick PCR purification kit (QIAGEN Inc., Chastworth, CA, USA) or E.Z.N.A. Clean kit (Omega Biotech, Doraville, USA). Both strands were sequenced separately using primers ITS1, ITS2, ITS3 and ITS4 with an ABI Prism 310 or 377 genetic Analyzer and the ABI Prism™ BigDye™ terminator Cycle Sequencing Ready Reaction kit with AmpliTaq®DNA Polymerase (Perkin Elmer Applied Biosystem). Navigator ™ Sequence Comparison software (Perkin Elmer) was used to identify the consensus sequence from the two strands of each ITS region. The new sequences have been lodged in the EMBL database with the accession numbers indicated in Table 2. Sequences obtained in this study were compared with homologous sequences of *Tulostoma beccarianum* Bresad. and *Chlamydopus meyenianus* (Klotzsch) Lloyd mentioned in MARTÍN *et al.* (2000) (EMBL GenBank Accession Numbers AF097752 and AF097755). Sequence Navigator™ Sequence Comparison for pairwise comparisons and SEQAPP software for multiple sequences were used to search for the best alignment. Where ambiguities in the alignment occurred, the alignment chosen was the one generating the fewest potentially informative characters. Alignment gaps were marked "-" and unresolved nucleotides or unknown sequences were indicated with "N". The data matrix was analysed using the PAUP*4.0 software package (SWOFFORD, 1998), under parsimony (MP) and maximum likelihood (ML). The polarity of characters was assessed with outgroup comparison, using *Tulostoma beccarianum* and *Chlamydopus meyenianus* as outgroup. In the MP analysis branch and bound algorithm was employed. In the ML analysis (FELSENTEIN, 1981) heursitic search and the HKY model of DNA substitution (KISHINO & HASEGAWA, 1989) were used. Branch robustness was estimated by bootstrap analysis (FELSENTEIN, 1985) of 1000 heuristic replicates.

**Table 1**. List of collections used in this study. In the first column the number is that which appears after the taxon name in Table 2 and Fig. 3 . (Pyr. Or.= Pyrénées Orientales)

| | | Herbarium | Collection date | Geographical origin |
|---|---|---|---|---|
| 1 | *B. guicciardiniana* | E, 809 Erb. Critt. Ital. Ser. II | 1878 | Italy |
| 2 | *B.phalloides* | LG | 20.08.1982 | Austria (North |
| 3 | *B.phalloides* | LG | 15.07.1982 | Pyr. Or., France |
| 4 | *B.phalloides* | LG | 02.07.1984 | Pyr. Or., France |
| 5 | *B.phalloides* | LG | 08.1981 | Pyr. Or., France |
| 6 | *B.phalloides* | LG | 06.08.1982 | Pyr. Or., France |
| 7 | *B.phalloides** | LG | 24.09.1978 | Hungary |
| 8 | *B.phalloides* | LG | 10.1981 | Mexico |
| 9 | *B.phalloides** | BCC-MPM 1866 | 1994 | Barcelona, S |
| 10 | *B.phalloides** | BCC-MPM1991 | 13.07.1995 | Barcelona, S |
| 11 | *B. phalloides** | MA-Fungi 15142 | 20.09.1978 | Hungary |
| 12 | *B. phalloides** | MA-Fungi 32085 | 12.10.1995 | Zaragoza, S |
| 13 | *B. stevenii** | LG | 19.06.1975 | Burundi |
| 14 | *B. stevenii* | LG | 05.11.1977 | Pyr. Or., France |
| 15 | *B. stevenii** | BCC-MPM 1990 | 13.07.1995 | Spain |
| 16 | *B.stevenii* | E, Wat herb. 10437 | 16.04.1974 | Australia, South |
| 17 | *B. stevenii* | MA-Fungi 34102 | 17.01.1995 | Gran Canaria, S |
| 18 | *B. stevenii** | MA-Fungi 29283 | 07.07.1992 | Tenerife, S |
| 19 | *B. stevenii** | MA-Fungi 31720 | 20.11.1992 | Jaén, S |
| 20 | *B. stevenii** | MA-Fungi 32476 | 13.11.1993 | Madrid, S |
| 21 | *B. stevenii* | MA-Fungi 32084 | 20.01.1994 | Madrid, S |
| 22 | *B. stevenii** | MA-Fungi 31008 | 20.08.1988 | Murcia, S |
| 23 | *B. stevenii* | MA-Fungi 22353 | 10.11.1987 | Madrid, S |
| 24 | *B. stevenii** | MA-Fungi 28224 | 03.05.1991 | León, S |
| 25 | *B. stevenii** | MA-Fungi 35883 | 05.10.1994 | Córdoba, S |
| 26 | *B. stevenii** | MA-Fungi 32477 | 20.11.1993 | Toledo, S |
| 27 | *B. stevenii** | MA-Fungi 31719 | 09.1990 | Madrid, S |
| 28 | *B.stevenii* | MA-Fungi 22354 | 2.4.1988 | Almería, S |
| 29 | *B.stevenii* | MA-Fungi 30681 | 6.8.1991 | León, S |
| 30 | *B.stevenii* | MA-Fungi 33554 | 17.6.1992 | Sevilla, S |
| 31 | *B.stevenii* | MA-Fungi 22357 | 23.11.1987 | Madrid, S |
| 32 | *B.stevenii** | MA-Fungi 27632 | 18.7.1990 | Mallorca, S |
| 33 | *B.stevenii* | MA-Fungi 22356 | 23.11.1987 | Madrid, S |
| 34 | *B.stevenii* | MA-Fungi 34099 | 8.11.1995 | Toledo, S |
| 35 | *B.stevenii* | MA-Fungi 22352 | 11.1987 | Segovia, S |

* Collections which sequences were obtained and submitted to the GenBank

**Table 2.** GenBank Accession Numbers for new nucleotide sequences obtained in this study and included in the phylogenetic analysis.

| | ITS 1 | ITS 1+5.8S+ITS2 | ITS 2 |
|---|---|---|---|
| *B.phalloides* (7) | | AF215654 | |
| *B.phalloides* (9) | | AF215656 | |
| *B.phalloides* (10) | | AF215657 | |
| *B.phalloides* (11) | AF090858 | | AF090859 |
| *B.phalloides* (12) | AF090856 | | AF090857 |
| *B.stevenii* (13) | | AF215655 | |
| *B.stevenii* (15) | | AF215658 | |
| *B. stevenii* (18) | | AF215648 | |
| *B.stevenii* (19) | | AF215649 | |
| *B. stevenii* (20) | AF090860 | | AF090861 |
| *B. stevenii* (22) | | AF215650 | |
| *B.stevenii* (24) | | AF215651 | |
| *B. stevenii* (25) | AF090862 | | AF090863 |
| *B. stevenii* (26) | AF090864 | | AF090865 |
| *B. stevenii* (27) | AF093703 | | AF093704 |
| *B. stevenii* (30) | | AF215653 | |
| *B. stevenii* (32) | | AF215653 | |

## RESULTS AND DISCUSSION

*Morphology.-* Among the 35 basidiomata examined, we observed a great variability in the basidioma size, even among specimens collected growing joined together (*B. phalloides* BCC-MPM 1991 and *B. stevenii* BCC-MPM 1990). It has not been possible to locate the type material of these two taxa in any of the herbaria consulted. Fig. 1 and 2 represent the morphotypes of *B. phalloides* (LG herbarium, leg. G.A. LaFuente, loc. Pyrénées Orientales, France, 15.7.1982) and *B. stevenii* (LG herbarium, leg. A. Marchan, loc. Pyrénées Orientales, France, 5.11.1977) that fit the protologues.

In the 35 basidiomata studied, the volva (Fig. 1b-c and 2b) hyphae form a loosely interwoven weft. The hyphae (7.2-8.5 µm diameter) are hyaline, septate and fibulate. No gelatinaized hyphae were observed in the volva of the *B. phalloides* collections. CALONGE (1998) studied two of these collections in fresh material (MA-Fungi 15142 and MA-Fungi 32085), mentioning the presence of the gelatinaized volva. It is interesting that the gelatinized hyphae in *B. phalloides* do not show up after drying which is uncharacteristic of gelatinaized hyphae in other fungi. Perhaps the gel of a fresh volva is of a different origin (Watling, per. comm.). CHERUBINI & GIAON (1997) after a study of numerous and repeated recoveries of basidiomes, state that the gelatinized volva is only produced in specific environmental conditions.

The *B. phalloides* elaters are narrower than in *B. stevenii* (5-5.8 µm diameter vs. 6.5-7.2 µm). The globose and sligthly verrucose spores are smaller in the first species (4.2-5.3 µm diameter vs. 5.2-5.6 µm diameter). Under SEM, spores appear finely verrucose with truncate warts or with anastomosing truncate ridges as described in BO & YIN-HUA (1983), but they are not related with the *B. phalloides* or *B. stevenii* morphotypes.

Demoulin (pers. comm.) has observed that in the two *B. stevenii* from LG herbarium there are not many hyphae in the inner part of the stipe and, moreover, these hyphae have a thick wall. However, we have been able to confirm this character but no differences were seen (Fig. 1e and

Fig. 2e), between *B. stevenii* and *B. phalloides* except the former has broader hyphae (14.5-17 3 μm diameter).

*Molecular analysis.*- The total aligned sequences ITS1-1 and ITS-2, including the 5.8S gene, were 722 base pairs (bp) long, of which 141 were parsimony informative. MP analysis (branch-and-bound search) gave two MPTs with a length of 382 steps, a consistency index (CI) of 0.8770 and a retention index (RI) of 0.9025. One of the MPT trees gave the same topology as the ML tree (-ln 2823.8535). Fig. 3 shows the consensus of the two MPTs.

Three main lineages appear in the phylogram, two of them are highly supported (100 % bootstrap value), although these three lineages are not related with any of the characters stated by early authors. The *B. phalloides* collections did not cluster together; they appear in the different lineages with the *B. stevenii* specimens. The two *B. phalloides* collections which CALONGE (1998) described as having the gelatinous volve (marked with "*" in Fig. 3) cluster in different groups. One typical of *B. stevenii* according to Dr. Demoulin (marked with "•" in Fig.3), cluster in one of the groups with four *B. stevenii* and one *B. phalloides.* However, the scanning photographs and the three lineages show that they have similarities in spore ornamentation (Fig. 3): *viz* (A) spores with anastomosing truncate ridges, (B) spores finely verrucose and (C) spore finely reticulate. Is this character enough to separate three species?

JACOBSON *et al.* (1999), based on 74 basidiomata, made an exhaustive study of the autoecology of *B. stevenii* in the ephemeral Kuiseb River in western Namibia. They predict that *B. stevenii* is a characteristic element of the riparian biota in dryland rivers. Perhaps the geographical distribution could be the point to separate the two taxa in this study. However, in our analysis, a collection from Burundi (marked with "•" in Fig.3) was included; it clusters between the other *Battarrea* collections from Europe.

The molecular data support the idea of Hollos (1904) that *B. phalloides* and *B. stevenii* belong to a single taxon, although Demoulin pointed to another possibility (pers. comm.): under other characters not described yet, different species could be separated. In our opinion, the spore ornamentation described here is not enough, neither is the stipe hyphae. Our conclusion, based on our morphological and molecular data, is that *B. phalloides* and *B. stevenii* could well belong to the same taxon. However, this only could be confirmed by studying the type material of both taxa which we have not been able to locate in any herbarium consulted. We would be happy to receive type or original material of these two fungi.

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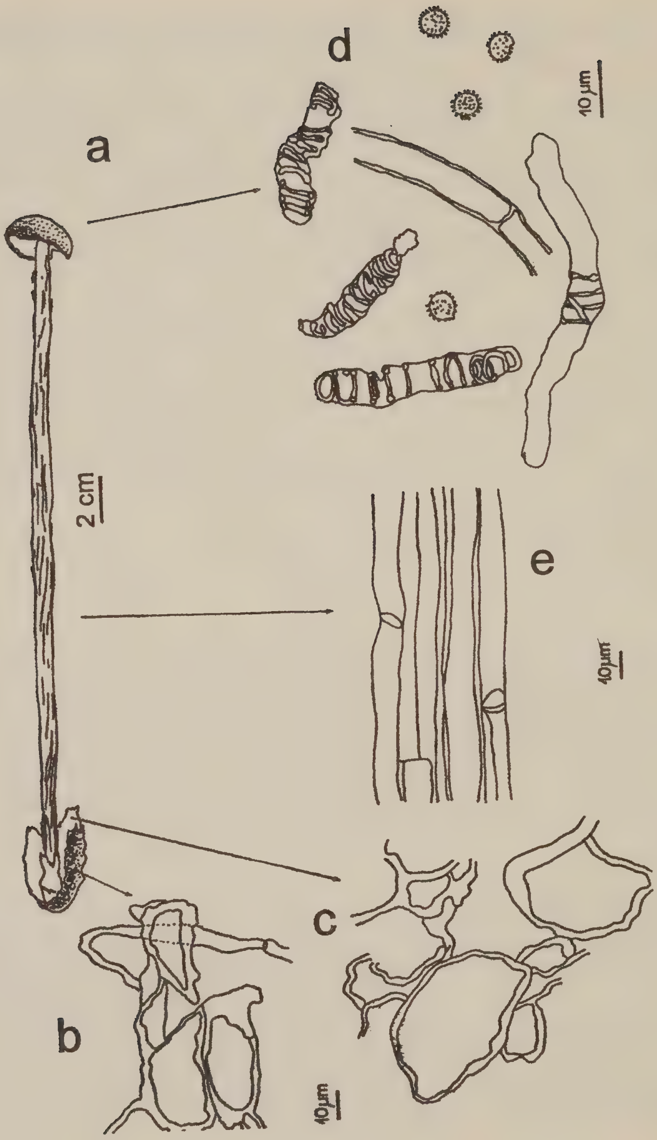

**Fig. 1** *Battarrea phalloides* morphotype, LG Herbarium (leg. G.A: LaFuente, Pyrénés Orientales, France, 15.07.1982). a) basidiome; b)-c) volva hyphae; d) elaters, capillitium and spores; e) stipe hyphae.

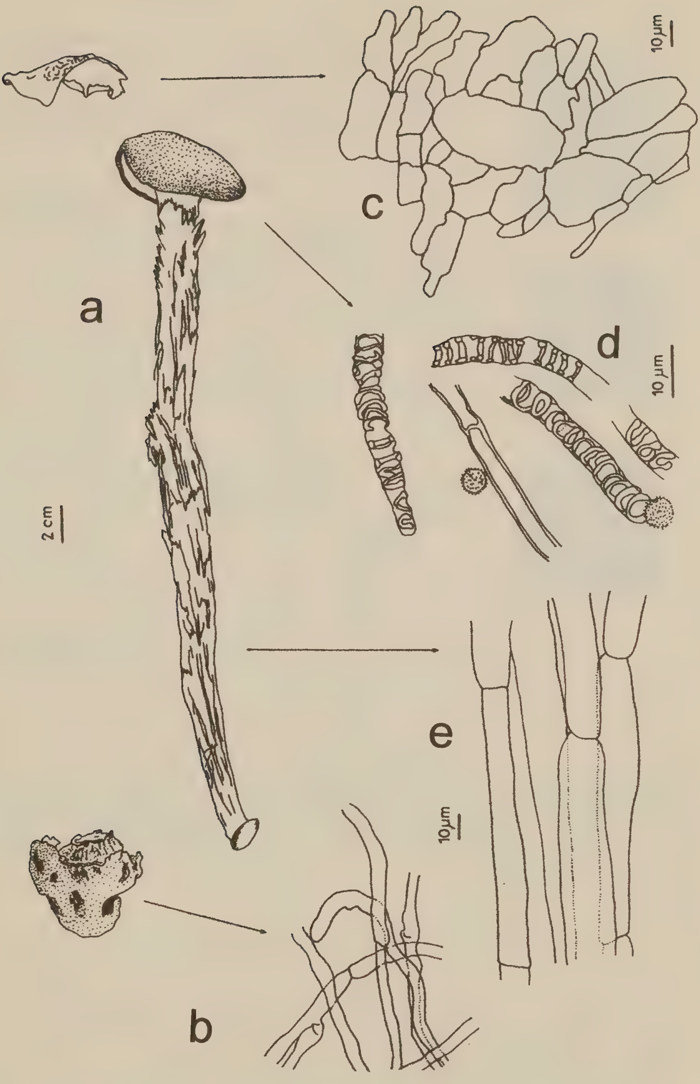

**Fig. 2** *Battarrea stevenii* morphotype, LG Herbarium (leg. A. Marchand, Pyrénés Orientales, France, 5.11.1977. a) basidiome; b) volva hyphae; c) peridium; d) elaters, capillitium and spores; e) stipe hyphae.

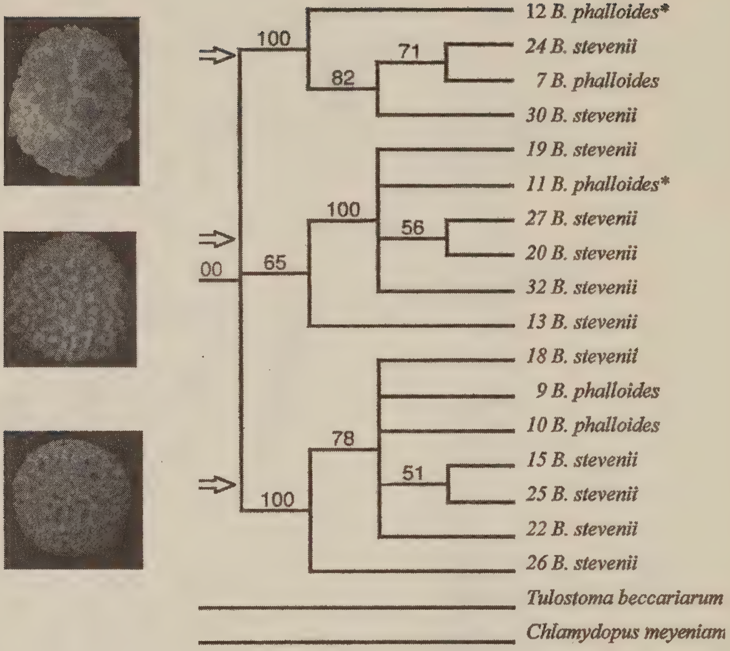

**Fig. 3** Molecular analysis of specimens included in Table 2 and sequences retrieved from the GenBank. Strict consensus of the two MPTs under branch-and-bound search using PAUP 4.0*. percentages from 1000 bootstrap replications are given on the branches (•: according to Dr. Demoulin typique *B. stevenii*; *: according to CALONGE (1998) collections with gelatinous volva in fresh conditions).

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# CYLINDROCLADIUM GORDONIAE SP. NOV.

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## ABSTRACT

*Cylindrocladium gordoniae* is described as a new species from leaf spots and blotches on *Gordonia lasianthus* in Florida.

Key Words: *Cylindrocladium gordoniae*, *Gordonia lasianthus*, leaf spot disease.

## INTRODUCTION

*Gordonia lasianthus* (L.) Ellis (loblolly-bay) is a flowering, evergreen shrub or small tree of the Theaceae. It inhabits moist lands from Virginia to Florida and Louisiana (Everett, 1981). The beautiful fragrant white flowers open in succession over an extended summer season (Everett, 1981). In October 1997, we found a species of *Cylindrocladium* sporulating on leaf spots and blotches of *G. lasianthus*. Plants were defoliating. Microscopic examination of the fungus revealed the presence of slightly bent conidia in addition to the straight conidia. The object of this study was to characterize the species found on *G. lasianthus* and compare its morphological features to those that have been reported to produce curved conidia, *C. curvatum* Boedijn &

Reitsma (Boedijn & Reitsma, 1950), *C. curvisporum* Crous & Victor (Victor *et al.*, 1997), *C. flexuosum* Crous (Crous *et al.*, 1995), *C. hawksworthii* Peerally (Peerally, 1991), *C. ovatum* El-Gholl, Alfenas, Crous & Schubert (Crous & Wingfield, 1994; El-Gholl *et al.*, 1993), *C. perseae* Schubert, Leahy, & El-Gholl (Schubert *et al.*, 1999), *C. pteridis* Wolf (Sobers, 1968) and *C. variabile* Crous, Janse, Victor, Marais & Alfenas (Crous *et al.*, 1993).

## MATERIALS AND METHODS

Leaf spots and blotches were surface-sterilized with 0.3% sodium hypochlorite for 3 min and plated on acidified potato dextrose agar (APDA, pH 4.3) to inhibit bacterial growth. The potato dextrose agar (PDA) was prepared as described by Schubert *et al.* (Schubert *et al.*, 1999). The *Cylindrocladium* from *G. lasianthus* was subcultured on peanut (*Arachis hypogaea* L.) stem-water agar (PS-WA) to enhance sporulation (Snyder & Hansen, 1947). PS-WA was prepared as described by Schubert *et al.* (Schubert *et al.*, 1999). Single-conidium isolates were obtained by the method of Hansen & Smith (Hansen & Smith, 1932). Single germinated conidia (12 curved and 12 straight) were transferred to APDA to compare colony morphology and pigmentation at 25 ± 2°C under laboratory conditions. Cultures grown on PS-WA were observed over a 6-week period.

Designation of pigmentation was expressed by the Munsell color codes (Munsell Color Company, Inc., 1975). In recording a color by Munsell notation, the symbol for hue is written first, and is followed by a symbol written in fraction form, the numerator indicating the value and the denominator indicating the chroma (H V/C). The hue notation of a color indicates a qualitative change. The value and chroma represent quantitative changes. The value notation indicates the lightness of a color; the higher the number, the lighter the color. The chroma notation indicates the strength of a color, increasing as the numerals increase.

Microscopic examinations and measurements were made on 72 primary, 74 secondary, 26 tertiary and 1 quaternary branches, 153 phialides, 262 conidia, 179 microconidia and 189 vesicles at X1000 under oil immersion lens. Vesicles were measured from the tip to the first septum and the widest vesicle width was recorded. All of the structures were mounted in water and were measured within 5 min of preparation to avoid swelling and distortion.

# RESULTS AND DISCUSSION

The culture from *Gordonia lasianthus* grew on APDA, PDA, and PS-WA. Upon subsequent subculturing, however, the isolate failed to grow on PDA; consequently, single-spore isolates were obtained from PS-WA.

Germinating conidia (12 curved and 12 straight) were compared on APDA at 25 ± 2°C. After 6 days, of the 12 curved conidia, only 4 formed colonies; 1 irregular in shape (17 mm across) and 3 round (27.7 mm average diameter). Of the straight conidia, 6 formed colonies; 3 irregular in shape averaging 17.6 mm across, and 3 round (28 mm average diameter). One round colony which sporulated best on PS-WA was submitted to the American Type Culture Collection as the ex holotype.

The selected type culture as viewed from the top showed a concentric pattern, with darker center, strong brown 7.5 YR 5/6, the middle ring was lighter in hue, reddish yellow 7.5 YR 6/6, and a white perimeter ring 7.5 YR 8/1 (9) (Fig. 1A). The bottom of the colony also showed a concentric pattern, a dark yellowish brown center 10 YR 3/4 surrounded by a very pale brown ring 10 YR 7/3, then a yellowish brown ring 10 YR 5/6 and a very pale outer ring 10 YR 8/3 (9) (Fig. 1B).

The species of *Cylindrocladium* from *G. lasianthus* has a single septate filament extending from the main axis of the conidiophore stalk terminating in a narrowly clavate vesicle (Fig. 1 C & D). Up to 4 serial branches are formed. Quaternary branches are seldom observed. Primary branches measure 18.2(11.9-26.7)x5.3(4-6.7)$\mu$m; secondary branches 14.6(8.9-20.2)x4.9(4-5.9)$\mu$m; tertiary branches 13.4(9.9-15.8)x4.7(4-5.5)$\mu$m; and 1 quaternary branch 13.9x4$\mu$m. Phialides are hyaline, doliiform 11.4(7.9-17.3)x4.7(4-5.5)$\mu$m. Conidia (Fig. 1 E & F) are granular, straight to slightly curved, 1(-3) septate, mostly (87.8%) 1-septate, 7.6% were 2-septate and 4.6% were 3-septate, 61.7(44.6-80.7)x5(4-6.2)$\mu$m. Septa were not always distinct. In older cultures (4-5 weeks), microconidia (straight, slightly bent or sinuous) were produced (Fig. 1G). They were mostly (97.8%) nonseptate and 1-septate, 1.1% were 2-septate and 1.1% were 3-septate. They measured 32.5(19.8-41.6)x3.6(3-4.2)$\mu$m. Cultures that were derived from single conidia yielded both conidia and microconidia on PS-WA. Vesicles were measured from the tip to the first septum. They were 62.6(21.8-122.8)x4.4(2.5-5.7)$\mu$m. Chlamydospores (Fig. 1 H & I) were produced on PS-WA within 4 weeks. This fungus did not form perithecia on PS-WA.

On the average, vesicles of *C. pteridis* are wider (7.9μm) (Sobers, 1968) than those of *C. flexuosum* (3.5μm) (Crous *et al.*, 1995), *C.* from *G. lasianthus* (4.4μm) and *C. curvatum* (5μm) (Boedijn & Reitsma, 1950). Phialides of *C. flexuosum* are longer (18μm) (Crous *et al.*, 1995) than those of *C.* from *G. lasianthus* (11.4μm). Conidia of *C. pteridis* are longer 87.5(61-118μm) (Sobers, 1968) than those of *C. perseae* 71.1(45.1-87.1μm) (Schubert *et al.*, 1999), *C. flexuosum* 73(50-80μm) (Crous *et al.*, 1995), *C.* from *G. lasianthus* 61.7(44.6-80.7μm) and *C. curvatum* (40-60μm) (Boedijn & Reitsma, 1950). Curved conidia are produced by *C. curvatum*, *C. curvisporum*, *C. flexuosum*, *C.* from *G. lasianthus*, *C. hawksworthii*, *C. ovatum*, *C. perseae*, *C. pteridis* (personal communication - P. W. Crous) and *C. variabile*. *C. curvatum* and *C. hawksworthii* have not been reported to produce microconidia. Microconidia are present in *C. flexuosum* (Crous *et al.*, 1995), *C.* from *G. lasianthus*, *C. perseae* (Schubert *et al.*, 1999) and *C. pteridis* (Crous *et al.*, 1993; Sobers, 1968). The filament extension in microconidiophores of *C. flexuosum* is absent (Crous *et al.*, 1995). The filament extension in microconidiophores of *C.* from *G. lasianthus* is present. The teleomorphs of *C. flexuosum* and *C. pteridis* are *Calonectria clavata* Alfieri, El-Gholl & Barnard and *Calonectria pteridis* Crous, Wingfield & Alfenas (Crous *et al.*, 1995; Crous *et al.*, 1993) respectively. *C. curvatum*, *C. hawksworthii*, *C.* from *G. lasianthus* and *C. perseae* are not known to produce the sexual state.

Based on the characteristics mentioned above, the *Cylindrocladium* from *G. lasianthus* is considered distinct from all *Cylindrocladium* spp. that have been reported to produce curved conidia, so a new species, *Cylindrocladium gordoniae* is proposed as follows:

*Cylindrocladium gordoniae* Leahy, Schubert *et* El-Gholl sp. nov.

Conidiophorum in vesiculam hyalinam, anguste clavatum, 4.4(2.5-5.7) μm latam terminans, usque ad 4 ramos seriatim efferens, ramis quaternariis raro observatis. Phialides hyalinae, doliiformes, 11.4 (7.9-17.3)x4.7(4-5.5) μm. Conidia recta vel leviter curvata, 1(-3)-septata, plerumque (88%) 1-septata, 61.7(44.6-80.7)x5(4-6.2)μm. Microconidia aseptata vel 1(-3)-septata, plerumque (98%) aseptata et 1-septata, 32.5(19.8-41.6)x3.6(3-4.2)μm.

| | |
|---|---|
| Habitatio: | seiunctum a maculis foliorum *Gordonia lasianthi*. |
| Locus typicus: | Green Cove Springs, Florida, U.S.A. |
| Specimen typicum: | FLAS F56790 |

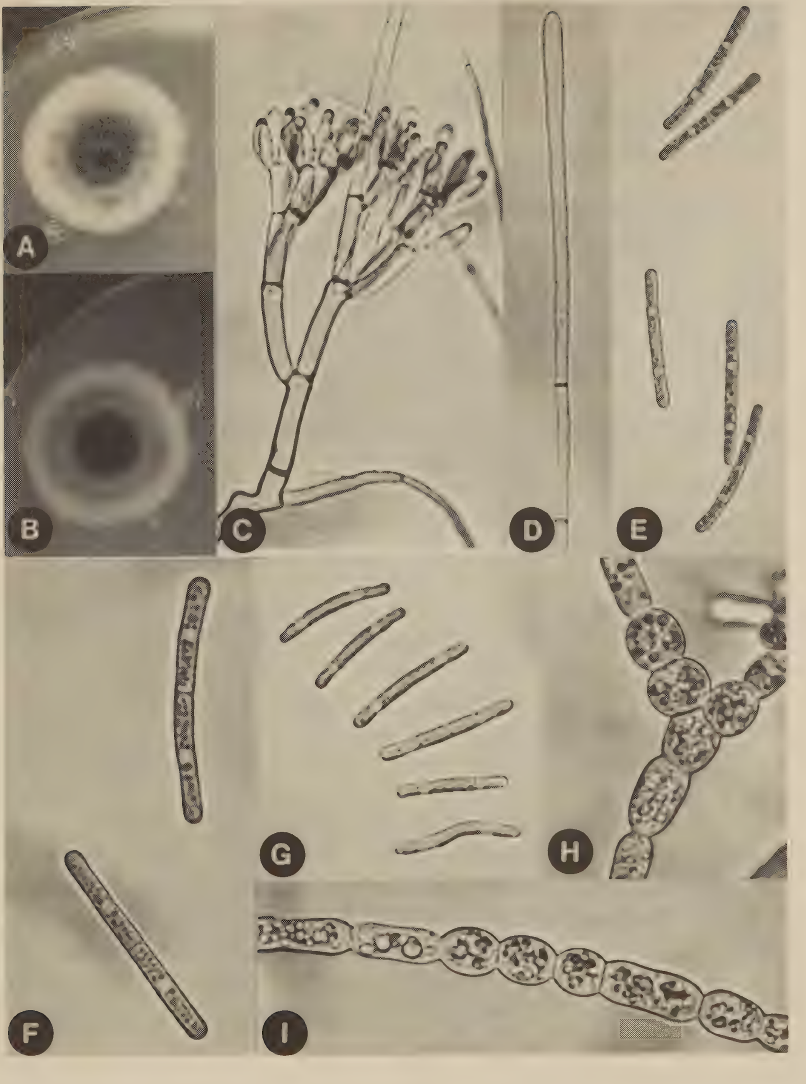

Fig. 1. *Cylindrocladium gordoniae*. A) Top of colony derived from a single conidium X0.9. B) Bottom of same colony X0.9. C) conidiophore showing a single central septate filament X640. D) Vesicle X640. E) Straight and slightly bent conidia X320. F) Conidia X640. G) Microconidia X640. H) and I) Chlamydospores X640.

Cultura typica: ATCC 201837

Lector: D. Chiappini
Datum: 17 Octobris 1997

*Cylindrocladium gordoniae* Leahy, Schubert and El-Gholl sp. nov.

Conidiophore terminating in a hyaline, narrowly clavate vesicle, 4.4(2.5-5.7)$\mu$m wide. Up to 4 serial branches are formed. Quaternary branches seldom observed. Phialides hyaline, doliiform, 11.4(7.9-17.3)x4.7(4-5.5)$\mu$m. Conidia straight or slightly curved, 1(-3) septate, mostly (88%) 1-septate, 61.7(44.6-80.7)x5(4-6.2)$\mu$m. Microconidia 1(-3) septate, mostly (98%) nonseptate and 1-septate, 32.5(19.8-41.6)x3.6(3-4.2)$\mu$m.

Habitat: Isolated from leaf spots and blotches on *Gordonia lasianthus*.

Type locality: Green Cove Springs, Florida, U.S.A.

Type specimen: FLAS F56790

Type culture: ATCC 201837

Collector: D. Chiappini
Date: October 17, 1997

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# TWO NEW HYPHOMYCETES FROM ARECACEAE IN LA GOMERA, CANARY ISLANDS

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## ABSTRACT

*Aquaphila edentata* anam. sp. nov. and *Neta tropicalis* anam. sp. nov, collected on decaying leaves of an unidentified Arecaceae in La Gomera, Canary Islands, are described and illustrated. The former is characterized by applanate conidiogenous loci and fusiform to subfalcate, 4(-5)-septate, hyaline conidia. The latter is distinguished by semi-circular, 0-1-septate, guttulate, hyaline conidia.

**Key Words**: *Aquaphila*, *Neta,* hyphomycetes, Arecaceae, systematics, tropical fungi, Spain.

## INTRODUCTION

Two interesting hyphomycetes were collected during a survey of microfungi from litter of rain forests in La Gomera, Spain. These fungi have morphological features of the genera *Aquaphila* Goh, K. D. Hyde & W. H. Ho (1998) and *Neta* Shearer & Crane (1971), but do not fit with any of previously published species of either genus and are described here as new.

## TAXONOMIC PART

***Aquaphila edentata*** R. F. Castañeda, Guarro *et* Gené, *anam. sp. nov.*

*Etymology.* Latin *edentatus*, without denticles. Figs. 1; 2 a,b

Ad fungus conidiales, hyphomycetes pertinentes. Coloniae in substrato naturali effusae, minutissime pilosae, albae. Mycelium plerumque superficiale et aliquot in substrato immersum. Hyphae septatae, ramosae, laeves, hyalinae, 1-2 µm diam. Conidiophora conspicua, mononematosa, simplicia, 0-2-septata, 10-35 × 2-3 µm,

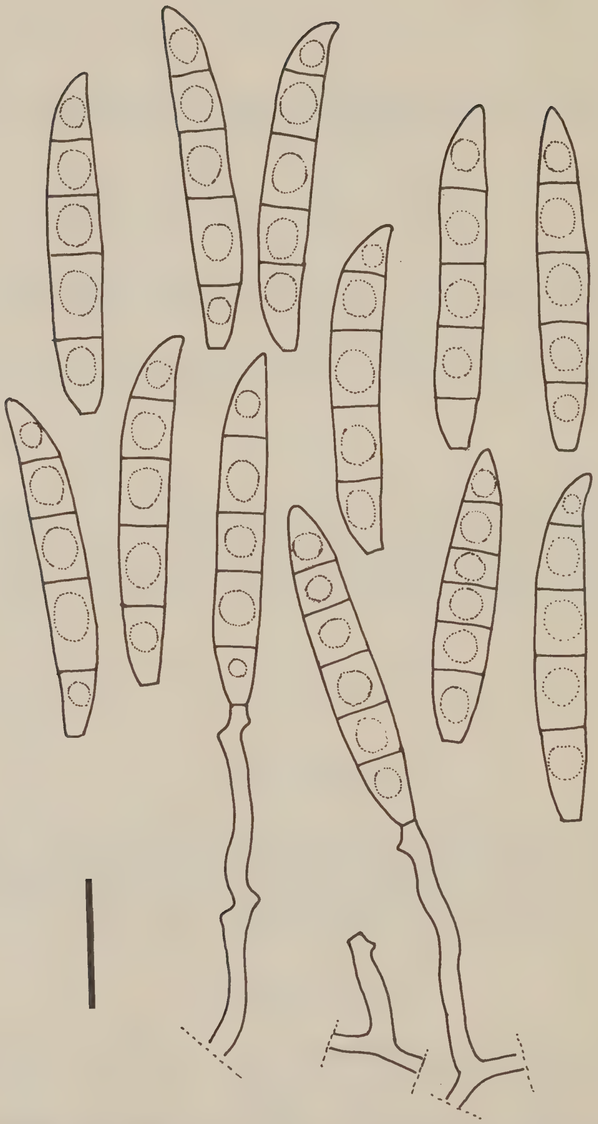

**Fig. 1.** *Aquaphila edentata,* MUCL 41846. Conidiophores, conidiogenous cells and conidia. Bar = 10 µm.

erecta, flexuosa, leviter geniculata ad apicem, levia, hyalina. Cellulae conidiogenae multiloculatae, hologenae, sympodialiter prolificae, edenticulatae, terminales, indeterminatae, hyalinae; in conidiophoris incorporatae. Loci conidiogeni applanati. Conidia fusiformia usque subfalcata, truncata ad basim, obtusa et leviter uncinata ad apicem, 4 (-5)-septata, 22-28 x 4-5 µm, plerumque guttulata, levia, hyalina; schizolytica secedentia. Teleomorphosis ignota.

*Matrix.* In foliis dejectis Arecaceae non determinata.

*Habitat.* In sylva pluvialis, La Gomera, Islas Canarias, 1000 m altitudis. Leg. J. Guarro, 20-VI-1999.

*Holotypus.* MUCL 41846.

Conidial fungi, hyphomycetes. Colonies on the natural substratum effuse, somewhat hairy, white. Mycelium superficial and immersed. Hyphae septate, branched, smooth, hyaline, 1-2 µm diam. Conidiophores conspicuous, mononematous, simple, 0-2-septate, 10-35 x 2-3 µm, erect, flexuous, slightly geniculate towards the apex, smooth, hyaline. Conidiogenous cells multilocal, hologenous, sympodially proliferating, non-denticulate, terminal, indeterminate, hyaline; integrated. Conidiogenous loci applanate. Conidia fusiform to subfalcate, truncate at the base, obtuse and slightly uncinate at the apex, 4(-5)-septate, 22-28 x 4-5 µm, guttulate, smooth-walled, hyaline, conidial secession schizolytic. Teleomorph unknown.

*Matrix.* On decaying leaves of unidentified Arecaceae.

*Habitat.* In a rain forest, La Gomera, Canary Islands, 1000 m altitude. Col. J. Guarro, 20 Jun 1999.

*Holotype.* MUCL 41846.

Colonies on OA at 25°C after seven days lanose, green, reverse olivaceous brown to dark green. No sporulation was observed.

*Cultures deposited.* MUCL 41846.

The genus *Aquaphila* is characterized by hologenous, unilocal or multilocal, sympodially proliferating, indeterminate, denticulate conidiogenous cells and solitary, multi-euseptate, fusiform to falcate or sigmoid, hyaline conidia that are truncate at the base. *Aquaphila edentata* resembles *A. albicans* Goh, K. D. Hyde & W. H. Ho (1998) in conidial shape, but the later has protuberant (denticulate) conidiogenous loci and hyaline to pale yellow, 45-80 x 8-9 µm, 6-14-septate conidia. The two species can be differentiated easily.

**Neta tropicalis** R. F. Castañeda, Gené *et* Guarro, *anam. sp. nov.*

*Etymology.* Latin *tropicus*, referring to the living place. Figs. 2 c,d; 3; 4

Ad fungus conidiales, hyphomycetes pertinens. Coloniae in substrato naturali effusae, albae. Mycelium plerumque superficiale, ex hyphis septatis, perramosis, laevibus, hyalinis vel subhyalinis, 1.0-1.5 µm diam. compositum. Conidiophora conspicua, mononemata, cylindrica, erecta, recta vel flexuosa, simplicia, raro ramosa, 0-1-septata, 8-30 x 2-3 µm, levia, hyalina vel dilute brunnea, interdum in cellula conidiogena reducta. Cellulae conidiogenae multiloculatae, hologenae, sympodiales, indeterminatae, hyalinae, in conidiophoris incorporatae. Conidiogeni loci longi denticulati, 1.5-3.0 µm longi. Conidia semicircularia, utrimque rotundata, 6-8 x 2-3 µm, biguttulata, interdum eccentrica inserta, solitaria, 0-1-septata, levia, hyalina, schizolytica secedentia. Teleomorphosis ignota.

*Matrix.* In foliis dejectis Arecaceae non determinatae et setis fungi non determinato.

*Habitat.* In sylva pluvialis, La Gomera, Canary Islands, Spain. Leg. J. Guarro, 20-VI-1999.

*Holotypus.* MUCL 41849.

Conidial fungi, hyphomycetes. Colonies on the natural substratum effuse, white. Mycelium mostly superficial, composed of 1.0-1.5 µm diam., septate, profusely branched, smooth-walled, hyaline or subhyaline hyphae. Conidiophores conspicuous, 8-30 x 2-3 µm, mononematous, cylindrical, erect, straight or flexuous, simple or rarely branched, 0-1-septate, smooth, hyaline or pale brown, sometimes reduced to conidiogenous cells. Conidiogenous cells multilocal, hologenous, sympodially proliferating, indeterminate, hyaline, integrated. Conidiogenous loci protuberant with several distinct denticles, 1.5-3.0 µm long. Conidia semi-circular, rounded at the ends, 6-8 x 2-3 µm, guttulate with two guttulae, solitary, 0(-1)-septate, smooth-walled, sometimes attached eccentrically, hyaline, schizolytically seceding. Teleomorph unknown.

*Matrix.* On fallen rotten leaves of unidentified Arecaceae species and on setae of unidentified fungus.

*Habitat.* In a rain forest, La Gomera, Canary Islands, Spain. Col. J. Guarro, 20 Jun 1999.

*Holotype.* MUCL 41849.

Colonies on PCA at 25ºC after ten days 10 mm diam., felted, grayish brown, reverse pale brown to brown. Hyphae septate, branched, hyaline or subhyaline, 0.5-2 µm diam. Conidiophores conspicuous, mononematous, erect, septate, pale brown below, subhyaline towards the apex, 35-80 x 3.0-3.5 µm. Conidiogenous cells sympodially proliferating, multilocal, indeterminate, integrated, denticulate with denticles cylindrical, 2-3 µm long. Conidia ovoid, slightly curved, 0-1-septate, hyaline, smooth-walled, solitary, guttulate, 6-11 x 2.5-3.0 µm, dry.

*Cultures deposited.* MUCL 41849 = CBS

The genus *Neta* Shearer & J. L. Crane (1971) includes species with hyaline, subhyaline, conspicuous, septate, simple or branched conidiophores which bear sympodially proliferating, integrated, indeterminate, hologenous, multilocal conidiogenous cells with long protuberant conidiogenous loci (denticles), and exhibit schizolytic conidial secession. The conidia are solitary, reniform, cylindrical, sublunate to allantoid, dumbbell-shaped or gibbous, 0- to several-septate, hyaline, and smooth. It includes terrestrial and aquatic species. The genus *Neta* comprises nine species: *N. patuxentica* Shearer & J. L. Crane (1971, the type species), *N. lignicola* Shearer (1974), the *N.* synanamorph of *Cladosporium bisporum* Matsush. (1975), *N. quadriguttata* (Matsush.) de Hoog (1985), the *Neta* synanamorph of *Ramichloridium ovoideum* (Matsush.) de Hoog, in Matsushima (1985), *N. compacta* de Hoog (1985), *N. salmonicolor* (Shearer) de Hoog (1985), *N. gibbosospora* R. F. Castañeda & W. B. Kendr. (1990) and *N. angliae* K. D. Hyde & Goh. (1999).

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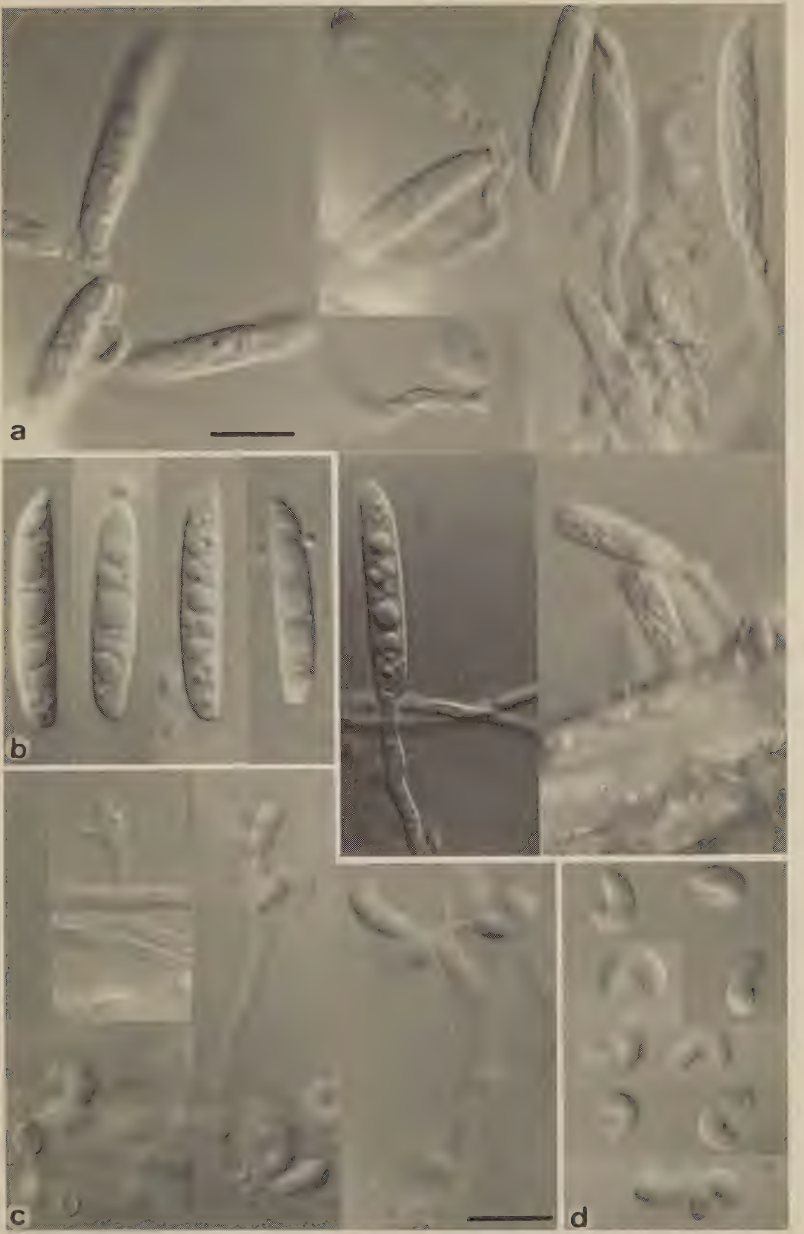
**Fig. 2.** a-b. *Aquaphila edentata*, MUCL 41846. a. Conidiogenous cells and conidia. b. Conidia. c-d *Neta tropicalis*, MUCL 41849. c. Conidiogenous cells. d. Conidia. Bars = 10 µm.


a
b
c
d

*Neta tropicalis* is superficially similar to *N. patuxentica* Shearer & Crane (1971), *N. salmicolor* (Shearer) de Hoog, and *Dactylaria fabiformis* Matsush. (1993) in conidial shape and septation. *Neta patuxentica* has 11-15 x 3-4 µm, reniform, 1-septate, hyaline and smooth-walled conidia; *N. salmicolor* has 9-12 x 3.5-4.5 µm, ovoid, 1-septate, guttulate, hyaline, smooth-walled conidia; whereas *D. fabiformis* has 13-18 x 3-4 µm, oblong, obtuse at the ends, 1-septate, guttulate, smooth-walled, hyaline conidia. *Neta lignicola* Shearer (1974) has 7-10 x 2-3 µm, unicellular, sublunate to allantoid, guttulate, curved, hyaline conidia and can also be easily distinguish from *N. tropicalis*.

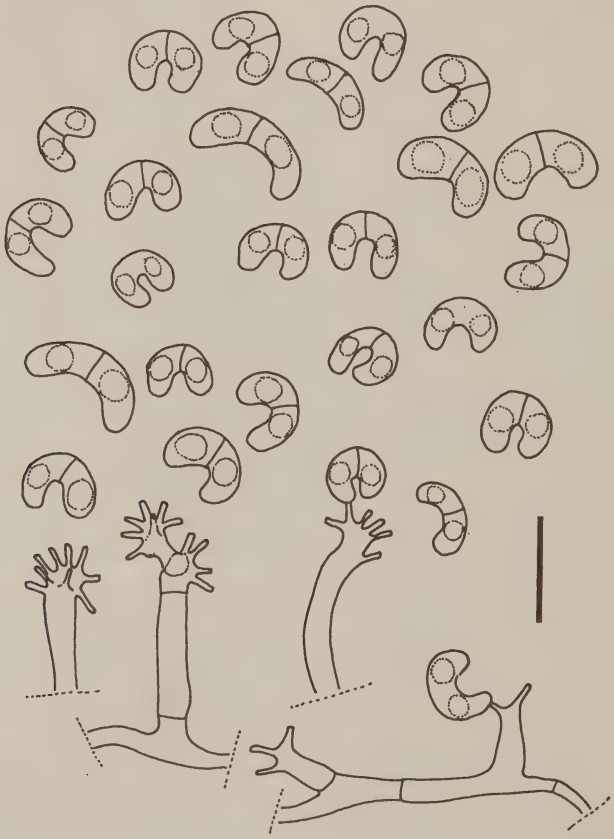
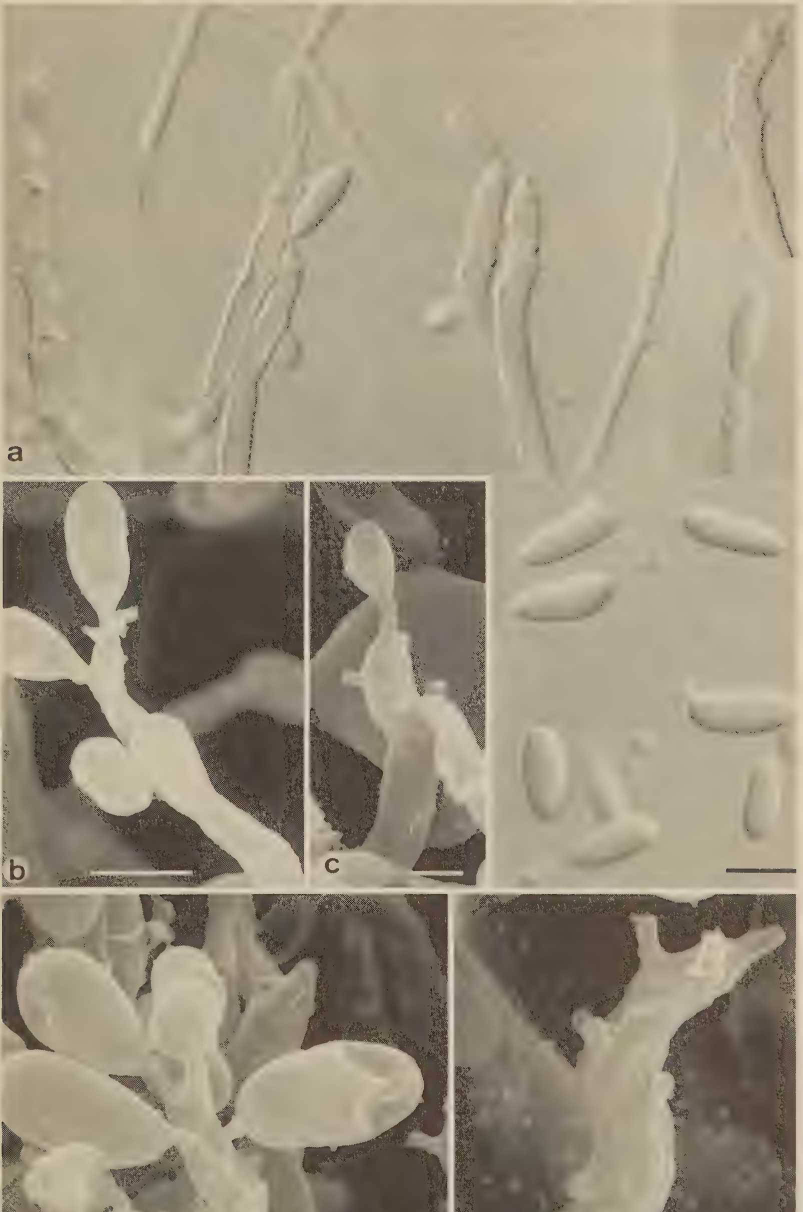


**Fig. 3.** *Neta tropicalis*, MUCL 41849. Conidiophores, conidiogenous cells and conidia. Bar = 10 µm.

**Fig. 4**. *Neta tropicalis* (MUCL 41849) ex culture on PCA. a. Conidiogenous cells and conidia. b-e. Conidiogenous cells and conidia (SEM). Bars: a = 10 µm, b = 5 µm, c-e = 2 µm.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# NEW SPECIES OF FUNGI FROM TROPICAL CHINA: A NEW SPECIES OF HYDROPISPHAERA (BIONECTRIACEAE)*

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**Abstract:** A new species of *Hydropisphaera* possessing discoid perithecia and fusoid, uniseptate ascospores is reported from tropical Guangdong, China.

**Key words:** *Hydropisphaera sinensis*; taxonomy.

A colorful disc-like pyrenomycete was encountered in our field excursion to the tropical region of Guangdong Province. It occurs on rotten leaf sheaths of bamboo and is a member of the Hypocreales. Judging by the discoid and relatively thick-walled ascomata, presence of subiculum at perithecial base, negative ascus iodine reaction, and uniseptate and fusoid ascospores, the fungus should be placed in the genus *Hydropisphaera* Dumort. of the Bionectriaceae which has recently been reviewed by Rossman *et al*. (1999). Among the existing species of *Hydropisphaera*, the Chinese material is similar to *H. gigantea* (Speg.) Rossman & Samuels recorded from South America in discoid perithecia and ascospore shape but differs from the latter in light orange- instead of dark orange-colored perithecia when fresh (dirty orange *vs*. black on drying) which are somewhat larger [250-450 μm wide and 200-300 μm high *vs*. (310-)340-370(-415) μm wide and (186-)230-360 μm high] and covered with hyaline instead of orange hairs, and shorter and narrower ascospores (38-50 x 5-6.4 μm *vs*. 48-55 x 6-7 μm) (Samuels, 1976). It represents a new species of the genus.

**Hydropisphaera sinensis** W. Y. Zhuang, Rossman & Samuels, **sp. nov.** (Figs. 1-6)

Ab *Hydropisphaera gigantea* peritheciis 250-450 μm diam, ascis 125-140 x 11.5-14 μm, ascosporis 38-50 x 5-6.4 μm differt.

Ascomata perithecial, scattered, superficial, seated on white subiculum, discoid to pulvinate, sessile, 250-450 μm wide across the flat top and ca 200-300 μm high, collabent when dry; light orange when fresh and dirty orange when dry, outer surface

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at flanks covered with whitish hairs, naked on the top and near the ostiole. Subiculum hyphae hyaline, with smooth to roughened walls. Hairs in fascicles, hyphoid, subcylindrical, slightly tapered towards the apex, hyaline, septate, 3-4 µm wide and up to 230 µm long or even longer, walls smooth. Ascomata hemispherical in longitudinal section, ascomatal walls ca 35-100 µm thick, thin at base and thick at flanks, interthecial cavity nearly spherical; cells in outer region of ascomata globose to angular, more or less isodiametric, subhyaline, thin-walled, 5-25 µm in diam; cells in the innermost region becoming ellipsoid to rectangular. Asci 8-spored, subfusoid to clavate, detectable only when young, pointed and with a narrow chamber-like structure at apex when young, walls extremely thin, J- in Melzer's reagent, ca 125-140 x 11.5-14 µm; walls deliquescing at maturity, sporiferous areas 89-102 x 10-13 µm. Ascospores elongate-fusoid with rounded ends, uniseptate, slightly constricted at septum, with 4-6 or more guttules, 3-4-seriate within ascus, walls smooth when young and becoming roughened when old, 38-50 x 5-6.4 µm. Anamorph not seen.

Holotype: Heishiding Natural Reserve, Fengkai County, Guangdong, China, alt. 300 m, on rotten leaf sheath of bamboo, 26-X-1998, Z. H. Yu & W. Y. Zhuang 2838 (HMAS 75513).

Etymology: The specific epithet refers to the type locality of the fungus.

## ACKNOWLEDGEMENTS

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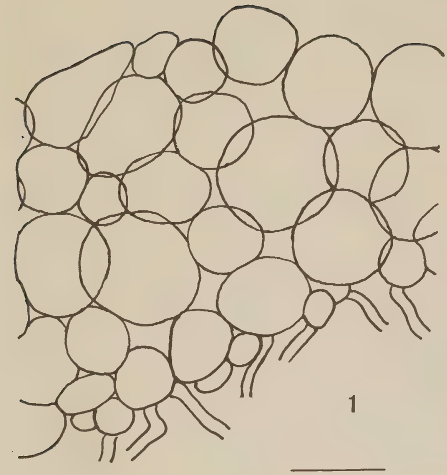
**Figs. 1-6**. *Hydropisphaera sinensis*: **1**. Structure in outer layers of ascoma with external hairs; **2**. Ascospores; **3**. Basal portion of a hair fascicle at the upper part of ascoma; **4**. Fragment of a subicular hair showing the roughened cell wall; **5**. Different ages of asci and ascospores, showing apical feature in young asci and a mass of mature ascospores with ascus wall not visible; **6**. Ascomatal anatomy. **1-5** scale = 20 µm; **6** x 45. All from HMAS 75513.



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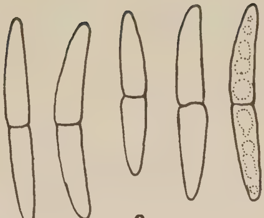

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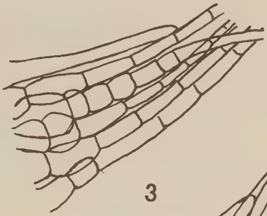

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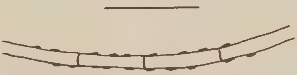

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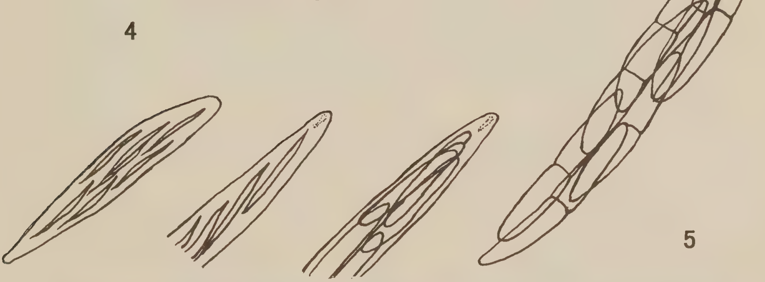

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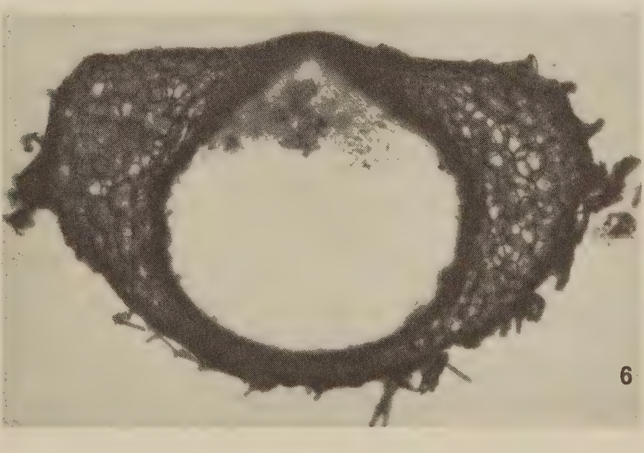

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# PLEUROTUS LINDQUISTII IS A LENTINUS

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**Abstract:** Using a strain determined by Singer 37 years ago as *Pleurotus lindquistii*, fruitbodies were obtained using traditional methods for edible mushroom culture. The new basidiomata allowed us to redescribe the species and to transfer it to *Lentinus*. Mating studies between monosporic cultures revealed a tetrapolar mating system. Descriptions of cultural characters and Nobles′ code are given.

**Keywords:** *Pleurotus*, *Lentinus lindquistii*, *Lentinus*, culture study.

## INTRODUCTION

To this time the knowledge of the genus *Pleurotus* in Argentina is scanty: only a few species have been recorded (Singer & Digilio 1955; Singer 1960, 1969; Raithelhuber 1974, 1977, 1991). In the fungus culture collection of our Biological Science Department we found a strain labeled *Pleurotus lindquistii* Singer. Singer (1960) described this species as growing on trunks of *Salix humboldtiana* in the marginal rain forest of Río de la Plata (Buenos Aires). The dikaryon strain was isolated by J. E. Wright two years later from specimens determined by R. Singer, collected in Pereyra Park, a large planted forest near La Plata city. This uncommon species was never collected again. Nevertheless we were able to obtain basidiomata from the dikaryon strain isolated in 1962. In the present work we redescribe and illustrate *Pleurotus lindquistii* Singer, transfer it to *Lentinus*, and report on its sexuality and cultural characters according to Nobles' methodology (Nobles, 1965).

## MATERIALS AND METHODS

**Basidiome production:** To obtain basidiomata traditional methods for fruiting species of *Pleurotus* were used (Zadrazil, 1974; Stamets, 1993). A mixture of sawdust (70 %), wheat meal (10 %), oatmeal (4 %) and $CaCO_3$ (1 %) was introduced in 40 x 25 cm polypropylene bags and autoclaved at 120 °C for 2 hours. After cooling they were inoculated with strain BAFC 2102, and incubated in the dark at 25°C. After 15 days, bags were kept at 18-20 °C with 9 h light/ 15 h darkness photoperiod to induce basidiome formation.

**Macro- and Micromorphology:** Specimens were macroscopically described. The description of this species combines cultures and herbarium specimens. Colour names are in accordance with Munsell color Co. (1954) and Rayner (1970). Citation of author names of taxa are according to Kirk & Ansell (1992). Herbarium abbreviations follow Holmgren *et al.* (1990). Specimens are deposited in the BAFC Mycological Herbarium. Microscopic examination of tissues mounted in 5% KOH and 1 % aqueous phloxine was undertaken.

**Pairing studies:** Monosporic cultures were obtained from water dilutions of fresh spore prints on sterile aluminum paper; spores were suspended in 10 ml of sterile-distilled water containing 0.01 ml of Tween 80 to avoid agglutination. This solution was diluted to 1/10, 1/100 and 1/1000. Petri dishes containing Nobles'medium (Nobles, 1948) were inoculated with 1 ml of each of these dilutions and incubated in the dark at 25°C. Colonies were reisolated in tubes, discarding all those having clamps. Haplonts were confronted in pairs in Petri dishes using 7-mm diam blocks as inoculum. Plates were incubated in the dark at 25°C, and after a week hyphae were observed under the microscope both in the contact zone and in the lateral area (Burnett, 1968). A positive result was reckoned (+) by the presence of clamps, and a negative one (-) by their absence.

**Culture characters:** Cultures were inoculated in 90 mm Petri dishes using Nobles' (1948) medium and incubated in the dark at 25°C. During six weeks, aspect, colour, growth rate, odour and microscopic structures of both the aerial and the submerged mycelium were observed weekly according to Nobles (1965).

Production of oxidases and phenoloxidases was evaluated by growing the strain on tannic and gallic media (Nobles, 1965). Tyrosine, p-cresol and guaiacol spots tests (Boidin, 1954), and the aniline test (Albertó & Wright, 1997) reactions were measured by the intensity of the colour halo produced. All the information thus obtained was codified in a "species code" according to Nobles (1965).

**Material studied:** ARGENTINA, Buenos Aires, Punta Lara, leg. Lindquist & Singer, 21-IV-60, BAFC (Holotype); Parque Pereyra, leg. R. Singer, 20-VI-62 (BAFC 34.673); San Isidro, Arroyo Sarandí, leg. J. C. Gamerro, 5-X-63 (BAFC) on trunk of *Salix*. Basidiomata obtained in culture: Llavallol, leg. E. Albertó & B. Lechner,12 IX-96 (BAFC 34.599); 29-IX-96 (BAFC 34.598); 2-II-96 (BAFC 34.597).

## RESULTS

***Lentinus lindquistii* (Sing.) Lechner & Albertó comb. nov.**
Basionym: *Pleurotus lindquistii* Singer, Bol. Soc. Arg. Bot. 8(3-4): 216. 1960.

Pileus 30-75 mm diam. (Fig. 1A), convex- cyathiform to slightly infundibuliform, white to cream, dry covered with squamules; squamules elongate, appressed or recurved, sepia to ochraceous brown (2.5YR 3/4, dark redish brown) fibrillose at the pileus margin and denser towards the centre; flesh thin, white to cream. Lamellae pale whitish (10YR 8/6; 8/8 yellow; Rayner 1-17d, paleoluteous) but pure white at the edges, fimbriate, subcrenulate, with lateral veins and anastomoses towards the stipe in young specimens, occasionally bifurcate, attenuate, subdecurrent, crowded, narrow. Stipe 10-40 x 5-11 mm, cylindric, large, tough, white to cream, yellowish, subcentral to eccentric, squamulose, densely covered with very small dark fibrils at the base (cream coloured in many specimens), that becoming larger, scattered and cream to yellowish towards the pileus, and with a pallid or whitish narrow evanescent cortinoid veil in the lower part in young specimens (Fig. 1B), absent in mature specimens, solid, in clusters. Odor strong, agreeable to slightly farinaceous, non-raphanoid. Spore print cream to whitish.

Basidiospores 6-7.5(-8) X 3.5-4 µm, Q= 2, oblong to oblong-cylindric, smooth, thin-walled, hyaline, inamyloid (Fig. 2C). Basidia 22-28 X 4.5-6 µm, 4-spored, clavate (Fig. 2D). Pleurocystidia absent or similar to basidioles. Cheilocystidia 12-35 X 3-6 µm, scattered, filiform, cylindric to clavate, hyaline. Hyphal pegs present (Fig. 2B).

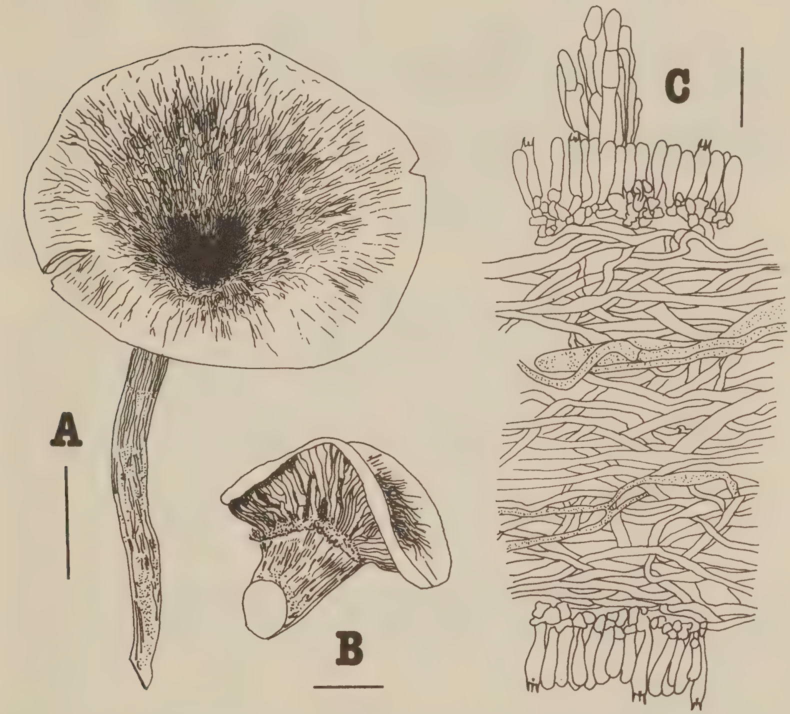

Fig. 1. *Lentinus lindquistii*. A, Basidiocarp; B, Young basidiocarp; C, Hymenophoral trama. Scale bar: Figs 1A = 1 cm, 1B = 0.5 cm, 1C = 25 µm.

Pileipellis a cutis, 20-30 µm thick; hyphae (Fig. 2A) radiating, clamped, usually thick-walled, non-gelatinized generative hyphae with brown citoplasmatic pigment. Hyphae of pileus (Fig. 2H) squamules clamped, filamentous, 2.5-7 µm diam.; skeletal hyphae (Fig. 2G) very scattered and scarce. Hymenophoral trama subregular, hiphae clamped, parallel to subparallel, non-gelatinous (Fig. 1C). Subhymenium poorly developed, 10 µm wide. Stipe tissue dimitic; generative hyphae, 3-4 µm diam.; skeletal hyphae 3-14 µm diam., 250-320 µm long, non-inflated, only tapered at the apex (Figs. 2F). Hyphae of stipe fibrils 2.5-7 µm diam., clamped, filamentous, with a brown pigment encrusting the walls.

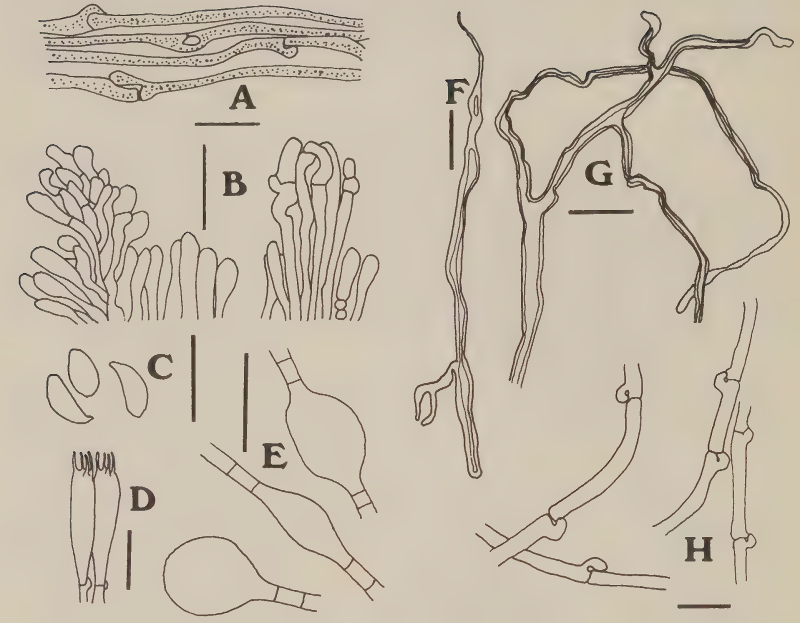

Fig. 2. Microscopical features of *Lentinus lindquistii*. A, Pileipellis; B, Hyphal pegs; C, Spores ; D, Basidia; E, Chlamydospores; F and G, Skeletal cells; H, Generative hyphae. Scale bar: Figs 2A = 30 µm; 2B, 2G = 20 µm; 2C, 2D, 2E, 2H = 10 µm; 2F = 40 µm.

**Mating system**

Eight subcultures were obtained. Pairing of these in all possible combinations revealed a tetrapolar mating system. Mating types were assigned as follows: isolates 3, 8= A1B1; 4, 5= A1B2; 2= A2B1 and 1, 6 and 7= A2B2 (Fig. 3).

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | |
| 2 | - | | | | | | | |
| 3 | + | - | | | | | | |
| 4 | - | + | - | | | | | |
| 5 | - | + | - | - | | | | |
| 6 | - | - | + | - | - | | | |
| 7 | - | - | + | - | - | - | | |
| 8 | + | - | - | + | + | + | - | |

Fig. 3. Shelf-cross grid for *Lentinus lindquistii*. +, compatible pairings; -, incompatible pairings.

### Culture characters

**Macroscopic Characters:** Mycelial mat covering Petri plates in 2-3 weeks, ocasionally with brown areas, whitish, loose near the inoculum, outward more appressed, cottony at the margin with abundant aerial mycelium; mycelial strands absent. Colony margin regular, entire . Reverse unstained. Odour mild, *sui generis*. No basidiocarps were produced during this period, since a light stimulus is required.

**Microscopic characters:** Hyphae hyaline (except brown in dark areas), clamped, 2-5 µm diam. Terminal and intercalary chlamydospores abundant, hyaline, 7-10 X 9-13 µm, increasing in number with age of culture. Microdoplets absent.

Oxidase reactions: Tannic acid strongly positive (++++); gallic acid strongly positive (+++++); p-cresol positive (+) without growth, tyrosine negative (-) with loose growth, aniline test (++) with loose growth.

Nobles species code: 2.3.7.34.36.(37).38.42.53.54.60.

## DISCUSSION

*Lentinus lindquistii* basidiomata are characterized by their relatively small size, presence of sepia-coloured fibrils on the cream-coloured pileus surface, fleshy basidiocarp, stipe tissue dimitic and presence of hyphal pegs on the lamellae.

Primordium development is characterized by a small spherical dark brown pileus and a relatively long stem densely covered with brown fibrils. Later, the pileus expands and as a consequence, the fibrils become more distant at the pileus margin and appressed and dense at the centre. Fibril colours are very variable, from dark brown to cream, depending on light regimes.

In young specimens, lamellae are distinguished by having pore-like anastomoses at the base of the stem (Fig. 4A); lamellae separate towards the pileus margin so they finally end as independent structures (Fig. 4B). The formation of reticulum did not occur in all specimens studied.

Skeletal hyphae are mainly present in the stipe trama as very long cells (Fig. 2B). Skeletal hyphae of pileus context are very scant and can only be observed after a detailed search.

Taxonomically, *Lentinus lindquistii* is close to *L. tigrinus* but differs from the latter by decurrent lamellae, sinuous clavate, often constricted or nodulose cheilocystidia and longer and narrower spores.

The generic delimitation of *Pleurotus*, *Panus* and *Lentinus* has been and still is controversial. Stankovičová (1973) made a comprehensive study of some of pleurotoid species of Agaricales showing the variaton existing among *Polyporus*, *Phyllotopsis*, *Pleurotus*, *Panus* and *Lentinus*, among other. She concluded that the hyphal system is not to be considered as a character of generic importance, but appeared to be fairly constant within genera such as *Pleurotus*, *Panus* and *Lentinus*, although both *Pleurotus* and *Lentinus* included both dimitic and monomitic hyphal construction.

Corner (1981), characterized *Lentinus* as follows: dimitic with skeleto-binding cells; hyphal pegs in most species; narrowly ellipsoid to subcylindric, more or less aguttate basidiospores; no pleurocystidia; *Panus* was diagnosed as: dimitic with long intercalary tapering skeletals cells; no hyphal pegs; broadly ellipsoid, guttate to subcylindric and aguttate basidiospores; pleurocystidia common, as metuloids or gloeocystidia. *Pleurotus* was defined as: monomitic or dimitic with terminal tapered skeletal hyphae; no hyphal pegs; basidiospores various, guttate or not, pleurocystidia ocasional. Corner (1981) pointed out that *Lentinus* could be clearly separated from *Pleurotus* but that the distinction between *Panus* and *Pleurotus* was obscure.

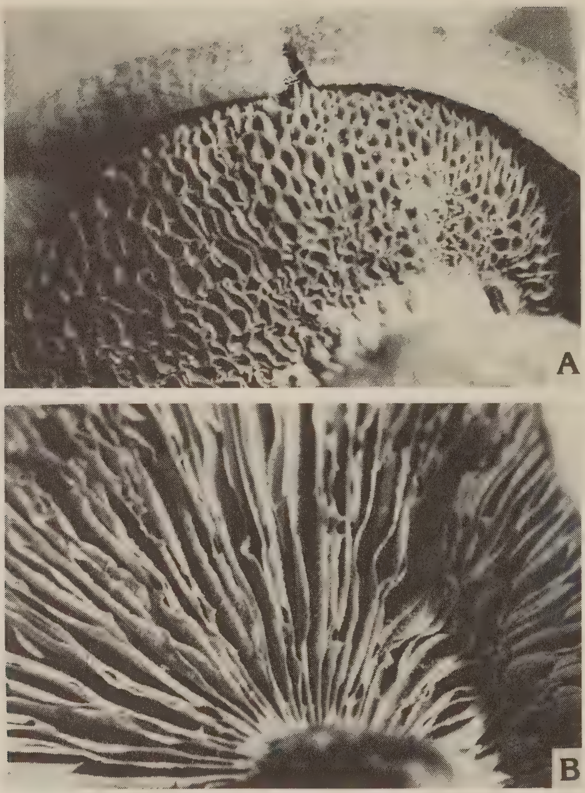

Fig. 4. *Lentinus lindquistii*. A, Anastomosed lamellae appearing as pores; B, Aspect of lamellae of mature specimens.

Pegler (1983) incorporated *Panus* within *Lentinus* and distributed its taxa at sectional level. He defined subg. *Lentinus* as having a context with skeleto-ligative hypae (i.e. thick-walled, intercalarly or terminal, branching laterally with tapering branches), hyphal pegs usually present, metuloids and gloeocystidia absent; and subg. *Panus* as having skeletal hyphae (i.e. thick-walled, typically unbranched; if branching is present, then not distinctly tapering), hyphal pegs absent; cystidia sometimes present.

Watling and Gregory (1989) accepted the genus *Lentinus* as outlined by Pegler (1983).

Singer (1975) placed *Pleurotus lindquistii* in sect. *Lepiotarii* (Fr.) Pilát of *Pleurotus*, which included species with a "lamellar trama at first consisting of thin-walled hyphae, eventually sclerified a dimitic at least in type specimens, veil present and a distinct stipe".

When mature basidiomata of *Lentinus lindquistii* have a soft coriaceus context which dries hard and rigid, subcentral to eccentric stipe, poorly developed subhymenium, irregular hymenophoral trama, hyphal pegs, dimitic hyphal system with tapered skeletal hyphae and, production of chlamydospores and absence of nematotoxic microdoplets in agar cultures. This latter character as suggested Miller (1984) and finally proposed Petersen (1993) can be used to segregate *Pleurotus* of other genera.

Following Pegler's (1983) concept, the above features support the transfer of this species to *Lentinus* Fr. subg. *Lentinus* (Fr.) Pegler (1983), section *Tigrini* Pegler (1983).

A culture study according to Nobles' methodology together with enzyme spot tests allowed the detection of laccases and phenoloxidases, warranting that the mycelia produce a white rot.

## AKNOWEDGEMENTS

We wish to thank Drs. R. Petersen (UT, USA), who suggested us that *Pleurotus lindquistii* could be a *Lentinus*, Dr. E. Horak (Swisserland) and J. E. Wright (UBA, Argentina) who kindly read the typescript and made valuable suggestions.

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# NOTES ON COPROPHILOUS BITUNICATE ASCOMYCETES

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## ABSTRACT

Comparison of variations in morphological features occurring in coprophilous bitunicate Ascomycetes leads to recognition of their relative importance. The presence or absence of germ pores or germ slits in ascospores, of variations in ascus apical anatomy, and of presence or absence of periphyses in the ostiole, in combination permit separation into three families: Phaeotrichaceae, Sporormiaceae, and the newly described Delitschiaceae.

The bitunicate coprophilous fungi and their relatives on decaying vegetative or woody substrates, usually in contact with soil, have many features in common. Their hypersaprotrophic nature and the darkly pigmented, usually strongly constricted ascospores are the most obvious ones. Features that separate these taxa tend to be more subtle.

Cain (1934) included assorted pyrenomycetous fungi in his study on Ontario species of coprophilous Sphaeriales. He carefully separated three classical bitunicate genera, *Sporormia* De Not. 1845, *Delitschia* Auersw. 1866, *and Pleophragmia* Fuckel 1870, in his key to genera by the method of ascus dehiscence "Ascus wall not stretching at maturity but rupturing by the breaking off of a thimble-shaped portion at apex allowing inner membrane to elongate." Since the 1950s two families have been recognized for bitunicate taxa, the Phaeotrichaceae Cain 1956 and the Sporormiaceae Munk 1957. Barr (1987, 1990b) arranged four genera as members of the Melanommatales. She assigned *Sporormiella* Ellis & Everh. 1892 to the Didymosphaeriaceae based on the presence of a thin clypeus; Ahmed and Cain (1972) did not consider that such a structure was present in this case. Barr assigned the other three genera, with obovoid to obpyriform ascomata and lacking clypei, to the Fenestellaceae. Barr (1987) had included the Sporormiaceae under Phaeotrichaceae as a synonymous name. She later (Barr 1990a) restricted the

Phaeotrichaceae to two genera with terminal germ pores in the ascospores, and considered that they represented unitunicate fungi and belonged in the Sordariales. Additional evidence and consideration have resulted in a different interpretation and in correction of several errors in disposition. The present study provides the evidence for an improved version in which three families are recognized and the subtleties of their variations are discussed.

The genera of concern are tabulated for important features that aid in their separation. The abbreviated notations include, for ascomata: perithecioid or cleistothecioid, ostiole none, pore, papilla, or periphysate; for asci: endotunica narrow, with well developed apical ring, or wide with refractive dome or rods; for ascospores: germ slits, terminal germ pores, or no germinal opening (0), septation (1-,3- etc.), other features such as separating into partspores at maturity, or wall surface ornamented.

| | ascomata: | | endotunica: | ascospores: | | |
|---|---|---|---|---|---|---|
| *Chaetopreussia* | cl | none | nar | 0 | 3- | sep |
| *Delitschia* | pe | per | rods | gs | 1- | |
| *Ohleriella* | pe | per | rods | gs | 3-13- | |
| *Phaeotrichum* | cl | none | nar | gp | 1- | |
| *Pleophragmia* | pe | pore | nar | 0 | 9- + 2 | |
| *Preussia* | cl | none | nar | gs | 3- | |
| *Pycnidiophora* | cl | none | nar | 0 | 3- | sep |
| *Semidelitschia* | pe | per | rods | gs | 0- | |
| *Sporormia* | pe | pore | nar | 0 | 15-31- | |
| *Sporormiella* | pe | pap | nar | gs | 3-7-9- | |
| *Spororminula* | pe | pap | nar | 0 | 7- | sep |
| *Sporormiopsis* | pe | pap | nar | gs | 3- | |
| *Trichodelitschia* | pe | per | ring | gp | 1- | |
| *Westerdykella* | cl | none | nar | 0 | 3- | orn,sep |

The genera tabulated are separable one from another with the exception of *Sporormiopsis* Breton & Faurel (Breton and Faurel 1964), which cannot be separated from *Sporormiella* Ellis & Everh. by the features utilized, as Hawksworth et al. (1995) and Eriksson and Hawksworth (1998) have already indicated. These genera are recognized as belonging to three families; the salient features that separate the families are utilized in the following key.

1. Ascospores with terminal germ pores; ascus endotunica narrow or with apical ring; ostiole periphysate or lacking Phaeotrichaceae

1. Ascospores with germ slits, or germinal mechanism lacking..... 2

    2. Ostiole not periphysate or ostiole lacking; ascus endotunica narrow, ocular chamber simple or with small apical dome or ring.............Sporormiaceae

2. Ostiole periphysate; ascus endotunica wide, ocular chamber containing refractive rods and often dome... Delitschiaceae

### *Phaeotrichaceae* Cain 1956

When Cain (1956) described the family he included only the cleistothecioid *Phaeotrichum* Cain & M.E. Barr, type species *P. hystricinum* Cain & M.E. Barr. This cleistothecioid genus is a much reduced taxon. Cain suggested that *Trichodelitschia* Munk 1953, type species *T. bisporula* (P. Crouan & H. Crouan) E. Müll. & Arx 1962, non validly published by Munk (1953) was closely related on features of the ascospores and the setose ascomata. Lundqvist (1964) assigned *Trichodelitschia* to the family, and described a second species, *T. munkii* Lundq. Parguey-Leduc (1974) demonstrated that *Trichodelitschia* showed pleosporalean development, and that the bitunicate asci contained a refractive apical ring. Barr (1990a) mistakenly considered these genera to be unitunicate ascomycetes and arranged the family in the Sordariales. I now recognize that the asci with well-developed refractive apical rings are truly bitunicate and fissitunicate. The hamathecium is composed of apically free paraphyses in *Trichodelitschia*. The Phaeotrichaceae shows features such as apical setae, periphysate ostioles, and cylindric asci with a delicate refractive apical ring in the apex. This combination of characters requires disposition as a separate family in the Melanommatales. At present only a few species are known in *Phaeotrichum* and two in *Trichodelitschia*.

### *Sporormiaceae* Munk 1957

The Sporormiaceae is included in the Pleosporales. The family contains both cleistothecioid and perithecioid genera; where an ostiole is present it is not periphysate. The ascus endotunica is narrow and the hamathecium may be composed of sparse pseudoparaphyses or in some taxa the sterile structures appear to be trabeculae. In some genera the ascospores bear germ slits, in others they do not; some separate into partspores at maturity. Several different sequences of genera are recognizable.

In the perithecioid species of *Sporormia* De Not. (synonyms *Brochospora* Kirschst. 1944, *Hormospora* De Not. 1844, p.p.), *Pleophragmia* Fuckel, and *Spororminula* Arx & Aa 1987, germ slits are not formed in the ascospores. *Sporormia* includes only a few taxa in the restricted sense of Ahmed and Cain (1972). The outer surfaces of the bundle of ascospores are thickened and dark whereas the inner adhering surfaces are pallid; the fascicle of ascospores is held together by a common gel coating. The dark outer wall and triangular shape of ascospores that readily separate into partspores were salient features utilized by Arx and Aa (1987) to erect *Spororminula*. In the sole species *S. tenerifae* Arx & Aa the ascospores do not form a close fascicle in the ascus; in other respects the genus may not be separable from *Sporormia*. In the dictyosporous *Pleophragmia leporum* Fuckel, each ascospore is cruciately longitudinally septate, the inner septa pallid in contrast to the dark outer surface; in vertical view each ascospore has the aspect of four and is surrounded by a gel coating. This genus is evidently closely related to *Sporormia*.

Several other taxa in the Sporormiaceae do not develop germ slits, the ascospores separate readily into partspores, and the ascoma is cleistothecioid. The small *Pycnidiophora* Clum 1955 (synonym *Preussiella* Lodha 1978) and *Westerdykella* Stolk 1955 have been combined and separated again (e.g., Cejp and Milho 1964, Arx 1981) under the slightly earlier name *Westerdykella*. Because of the spirally ornamented ascospore walls in *W. ornata* Stolk (Stolk 1955), an unusual feature within the family, I prefer to maintain separate genera with *Pycnidiophora* recognized as forming smooth-walled ascospores (Clum 1955). *Chaetopreussia* Locq.-Lin. (Loquin-Linard 1977) is unknown to me but the description of setose ascomata would in theory set it apart from *Pycnidiophora*.

Two genera in the family do exhibit germ slits in the ascospores. *Preussia* Fuckel 1867 (synonyms *Fleischhakia* Auersw. 1869, *Honoratia* Cif., Vegni & Montemart. 1963) is typically nonostiolate and the asci are broadly clavate and stipitate (Cain 1961). Some small ostiolate species have been inserted in *Preussia*. Examples include *Sporormia minima* Auersw., as *Sporormiella minima* (Auersw.) Ahmed & Cain, *Preussia minima* (Auersw.) Arx, and *Sporormiopsis minima* (Auersw.) Breton & Faurel; or *Sporormiella sorghophila* (Peck) M.E. Barr and *Preussia sorghophila* (Peck) M.E. Barr. Some difficulty will be apparent in assigning certain species with germ slits to genus: I suggest that oblong or cylindric asci with short or no stipes may serve to designate *Sporormiella* when ascomata open by a pore or small papilla. Both of the species used as examples are thus species of *Sporormiella*. *Sporormiella* is here accepted for ostiolate taxa, much as delimited by Ahmed and Cain (1972), rather than as Barr (1990b) understood the genus. The type species *S. nigropurpurea* Ellis & Everh. is not strongly clypeate and the short papillate ascomata without periphyses, with a narrow peridium, and oblong, short stipitate asci in sparse pseudoparaphyses (trabeculae?) is recognized now as part of the variation within this genus. *Sporormiella oblongiclavata* M.E. Barr & Malloch is a wood-inhabiting species in the genus, close to *S. octomera* (Auersw.) Ahmed & Cain, but with ascospores having the second rather than third cell enlarged and the germ slits nearly straight rather than diagonal. The third species that Barr (1990b) assigned to the genus, *S. subcongrua* (Ellis & Everh.) M.E. Barr has light reddish brown ascospores with pallid end cells and lacks both germ slits and deep constrictions at the septa. This species appears to be better assigned to *Trematosphaeria*. Certain other species presently assigned to *Sporormiella* may well belong to *Ohleriella*, just as *Sporormia herculea* Ellis & Everh. [*Sporormiella herculea* (Ellis & Everh.) Ahmed & Cain = *Ohleriella herculea* (Ellis & Everh.) M.E. Barr] does. The genera belonging to the Sporormiaceae may be recognized by the features emphasized in the following key.

1. Ascospores with germ slit in each cell..... 2
1. Ascospores lacking germ slits..... 3
    2. Ascomata cleistothecioid; asci widely clavate and usually stipitate..... *Preussia*
    2. Ascomata perithecioid; asci oblong to cylindric, short stipitate or sessile..... *Sporormiella*
3. Ascospores triangular in transverse view; ascomata perithecioid..... 4
3. Ascospores rounded in transverse view, 3-septate, readily separating into partspores; ascomata cleistothecioid..... 6
    4. Dictyosporous, septation 9 + 2, inner surfaces pallid.... *Pleophragmia*
    4. Phragmosporous..... 5
5. Ascospores 15-31-septate, inner surfaces pallid..... *Sporormia*
5. Ascospores 7-septate, inner surfaces nearly as dark as outer, readily separating at maturity..... *Spororminula*
    6. Ascospore walls ornamented by spiral bands..... *Westerdykella*
    6. Ascospore walls smooth..... 7
7. Ascomata glabrous or with hyphal appendages..... *Pycnidiophora*
7. Ascomata setose..... *Chaetopreussia*

### *Delitschiaceae* M.E. Barr, fam. nov.

Similis vel Sporormiaceae praeter ostiolum periphysatum et endotunica asci lata cum virga refractiva in oculo. Typus familis: *Delitschia* Auersw.

Ascomata medium to large sized, apex papillate; ostiole periphysate. Hamathecium of trabeculae in matrix. Asci with wide endotunica, ocular chamber surmounted by dome and usually containing four refractive rods. Ascospores strongly pigmented, unicellular or septate, each cell containing germ slit, surrounded by gel coating. Hypersaprotrophic on old dung or exposed wood.

Three genera are included here, recognized according to ascospore septation.

1. Amerosporous..... *Semidelitschia*
1. Septate, readily separating into partspores..... 2
    2. Didymosporous..... *Delitschia*
    2. Phragmosporous..... *Ohleriella*

These melanommatalean genera differ from both of the earlier described families. Munk (1957) had already recognized that *Delitschia* was distinctive in the presence of well developed, periphysate ostioles in the ascomata, and mentioned but did not specify the nature of the 'different' ascospores. Arx and Aa (1987) did not include *Delitschia* and its relatives when they supplied an emended key to genera in the Sporormiaceae, but Arx and Müller (1975) inserted both *Delitschia* and *Semidelitschia* in their key to Sporormiaceae.

Cain and Luck-Allen (1969) described the ascus apex in *Semidelitschia agasmatica* Cain & Luck, the sole species. They noted similarity in this feature to species of *Delitschia*. The wide endotunica and ocular chamber in the ascus apex was well illustrated by Luck-Allen and Cain (1975) especially for *Delitschia pachylospora* Luck-Allen & Cain (fig. 157) and *D. patagonica* Speg. (figs. 153, 158, 159), and by Jeng et al. (1977) for *D. dochmiophragma* Jeng, Luck-Allen & Cain (fig. 5) and for *D. mesostenospora* Jeng, Luck-Allen & Cain (fig. 19). I have only recently realized (Barr 1987, 1990b) that the presence of a set of refractive rods extending downward from the ocular chamber serves as another valid feature in ascus anatomy, although Eriksson (1981, fig. 3c) illustrated and commented on these structures. He observed that Chadefaud (1942) first described the complications of apical nasse.

The melanommatalean group of coprophilous or wood-inhabiting genera is typified by *Delitschia* Auersw. (synonyms *Delitschiella* Sacc. 1905, *Pachyspora* Kirschst. 1906); for species see Cain (1934), Luck-Allen and Cain (1975), Jeng et al. (1977). *Semidelitschia* Cain & Luck is monotypic with only *S. agasmatica* Cain & Luck (Cain and Luck-Allen 1969). Barr (1987, 1990b) revived *Ohleriella* Earle 1902 for otherwise similar but phragmosporous taxa, and arranged these genera in the Fenestellaceae. However, the trophic states are quite different between the two groups of fungi: in the Fenestellaceae semibiotrophic to saprotrophic on woody branches or stout herbs, either dicot- or monocotyledonous, in the Delitschiaceae hypersaprotrophic on old dung or aged exposed wood. It seems reasonable to present a separate family now, rather than to propose a subfamily that in all likelihood would soon be raised to family status.

It is a pleasure to acknowledge Clark T. Rogerson for kindly reviewing the manuscript.

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# CORTINARIUS VERNICOSUS: A NEW SPRING SPECIES OF SUBGENUS MYXACIUM FROM WASHINGTON STATE

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***Abstract:*** *Cortinarius vernicosus*, a new spring species in mature to late successional conifer forests, is described from two disjunct localities of Washington State in western North America. It is a member of subgenus *Myxacium*, section *Myxacium*, due to its cylindrical stipe and the presence of clamp connections throughout the sporocarp. The relationship of *C. vernicosus* to other members of section *Myxacium*, based on morphological and phylogenetic data, is discussed. Vegetation, geology and soils are compared and contrasted for the two localities. With this addition, there are six species of section *Myxacium* known from western North America.

***Key Words:*** Agaricales, Basidiomycetes, Cortinariaceae, macrofungi, taxonomy.

## Introduction

The mature to late successional conifer forests of western North America are rich in *Cortinarius* species. In this region most of the ectomycorrhizal species produce sporocarps during the late summer and fall seasons, with fewer fruiting in the spring (Ammirati et al. 1994; Odell et al. 1999). Similarly in northern Michigan, Lussenhop and Fogel (1999) reported that 85% of the ectomycorrhizal species they studied produced sporocarps in the fall season. At higher elevations, fruiting begins later in the spring depending on the snowpack and temperature. Among the *Cortinarius* species that occur during spring and early summer in western North America are: *C. zinziberatus* (Fr.) Fr., *C. clandestinus* Kauffm., *C. erythrinus* (Fr.) Fr. and *C. croceus* (Schaeff.) Bigeard & Guillemin. *Cortinarius trivialis* J. E. Lange (subgenus *Myxacium*, section *Myxacium*) occurs during late spring to summer in association with *Populus* in western North America. *Cortinarius vernicosus* represents a second spring-fruiting species in subgenus *Myxacium*, section *Myxacium*. Species in this section produce hyphae with clamp connections, while those in the morphologically similar section *Defibulati*, lack clamp connections. The two sections are also distinguished from each other by their pattern of basidiospore ornamentation when observed with SEM, and a well-defined mediopellis layer is present in the pileipellis of members in section *Defibulati*.

*Cortinarius vernicosus* was first discovered in Olympic National Park (ONP), in old-growth *Pseudotsuga menziesii/Tsuga heterophylla* forests of western Washington by T. O'Dell (TOD). Subsequently it was discovered by the author in late successional mixed conifer forests of northeastern Washington (Fig. 1). This species appears to be associated with *Pseudotsuga menziesii* (Mirb.) Franco, in a disjunct distributional pattern. Field studies since 1992 have resulted in six known general localities from the northwest, and one locality from the northeast portion of the state. This taxon apparently does not fruit during the fall season. No specimens of *C. vernicosus* were found in the University of Washington Herbarium (WTU) with collection dates earlier than 1992.

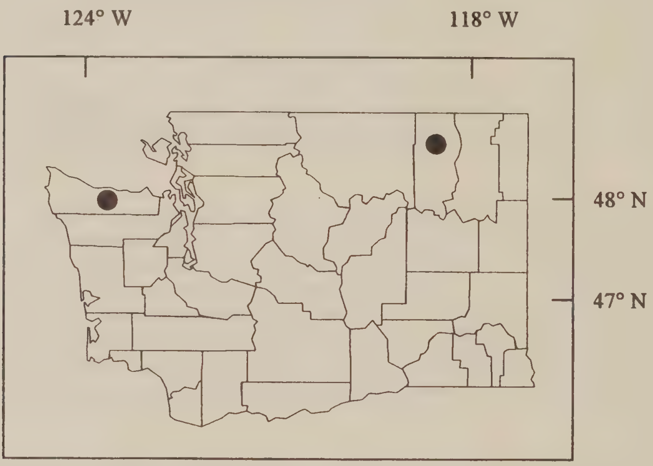

**Fig. 1.** Map of Washington state showing the two general localities for *Cortinarius vernicosus*. Map by permission from D. L. Largent (1994).

The western ONP localities produce sporocarps beginning in April, particularly in May, and into June (Fig. 2). At higher elevation localities from Sherman Pass in eastern Washington, sporocarp production begins in June and continues into July (Fig. 2). According to Dr. Meinhard Moser (personal communication), no comparable species occurs in Europe, nor has a similar species been reported in the literature.

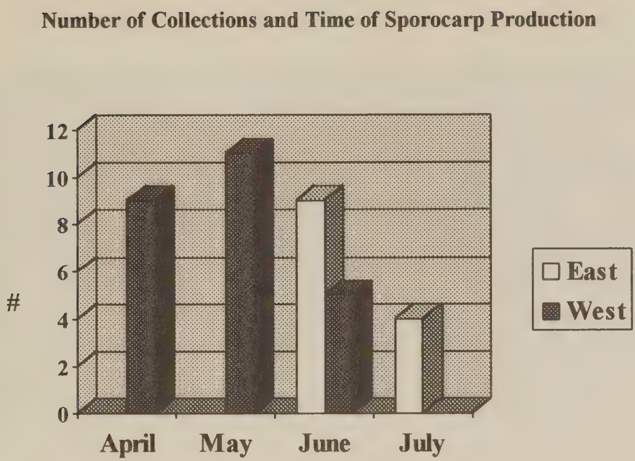

**Fig. 2.** Graph showing number of collections examined and their phenology. Eastern collections are from Sherman Pass, Colville N. F., and western collections are from Olympic N. P., WA.

## Materials and Methods

Complete macromorphological descriptions of fresh collections form the basis of this taxonomic study, supplemented by notes and collections from other workers, for a total of 38 cited collections. The collections are deposited at WTU, except where indicated under collections examined using notations following Holmgren et al. (1990). Specific color designations are as follows: Ridgway (1912), (Prout's Brown), Kornerup and Wanscher (1978), maize yellow (4A6), and Munsell (1954), 7.7YR 7/4 (pink). Generalized colors are those of the author and regarded as useful approximations.

Chemical reactions were made using 3% KOH and Melzer's reagent applied to the pileus and stipe surfaces and context.

Microscopic examination of fresh material was in water and/or 3% aqueous solution of KOH. Dried material was examined using 3% KOH and Melzer's reagent. A random selection of 35 (minimum) mature spores obtained from spore prints or from stipe/cortina surfaces were studied from each collection. Free-hand radial sections of the pileus were used to study pileipellis structure. Letter abbreviation for $Q$ = mean length/width ratio of basidiospores.

Scanning electron photomicrographs of abaxial and adaxial views of mature basidiospores are included (Figs. 3-4). Dried basidiospores from spore prints of stipe/cortina surfaces were coated with gold/palladium in a Technics Hummer V sputter-coater and photographed using a JEOL JSM-840A scanning electron microscope.

## Taxonomy

***Cortinarius vernicosus*** Seidl, sp. nov. Figs. 5-6

Pileus 0.8-4.3 cm diametro, obtuse conicus vel convexus, denique umbonatus, glutinosus, glaber, hygrophanus evadens, glutine incolori, margine languide striato, pallide ochraceo-luteo, plerumque disco obscuriori, in brunneum evadente, ochraceo-aurantiaco vel luteo-brunneo colore olivaceo tincto. Lamellae roseo-luteae, pallide griseae, vel plerumque novae violaceae, in colorem luteo-brunneum vel brunneum evadentes, adnatae vel adnexae, nonnumquam maturiores parum subdecurrentes.

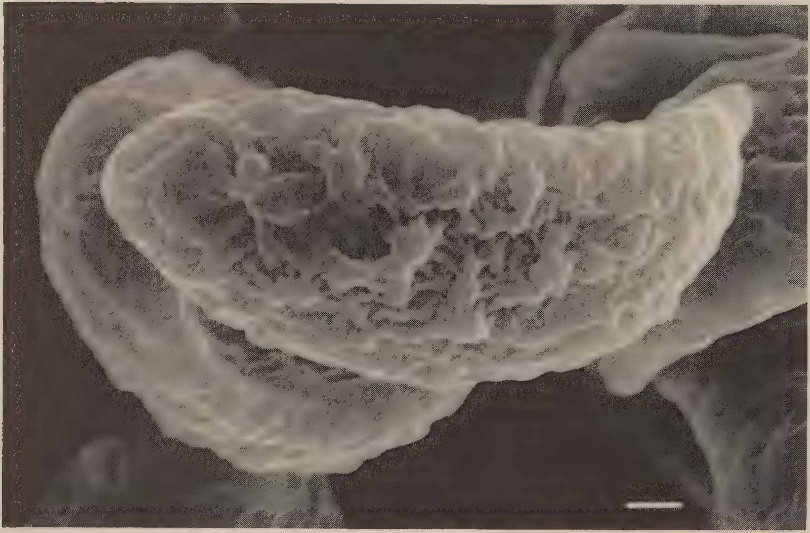

**Fig. 3.** Basidiospore of *Cortinarius vernicosus*, abaxial view, MTS3703. Scale bar = 1μm.

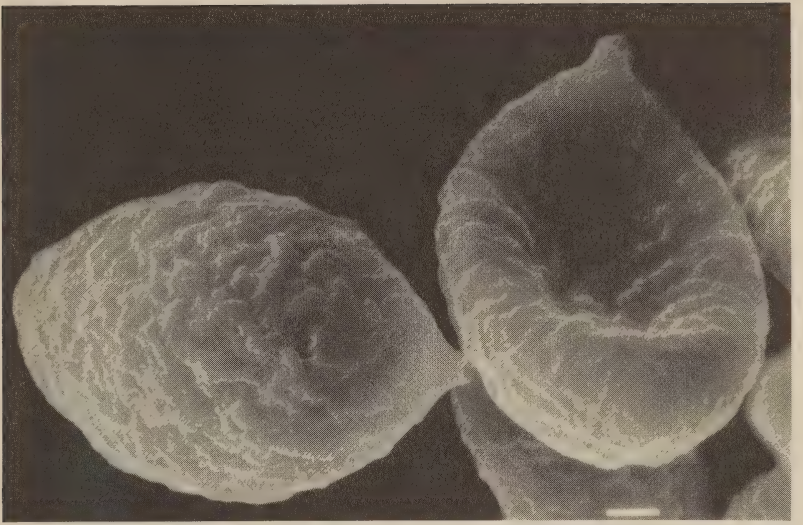

**Fig. 4.** Basidiospore of *Cortinarius vernicosus*, abaxial and adaxial views, MTS3704. Scale bar = 1μm.

Stipes 2.7-11.7 cm longitudine, ad apicem 1.5-8 mm diametro, 3-10 mm ubi maxime crassus, cylindricus vel subfusoides, superficie superiori alba, inferiori pallide lutea vel cremea, glutinosa, velo interiori albo, interdum in areolas irregulares diffracto, stipitis basis contextu ochraceo vel brunneo. Basidiosporarum copia colore brunneo luti vel tabaci, sporae 9.4-15.9 um, amygdaliformes vel limoniformes, verrucosae. Cystidia nulla, zygodesmata observata. Solitarii vel sparsi, sub *Pseudotsuga menziesii* vel arboribus coniferis mixtis. Holotypus USA, WA, Sherman Pass, Colville Silva Nationalis, 1 July 1995, legit M. T. Seidl, *MTS3702* (WTU).

*Pileus* (8-)10-43 mm diam, at first subconical to narrowly convex with a slightly inrolled margin, becoming broadly conical or broadly convex with an umbo, rarely subpapillate, eventually broadly umbonate with upturned margin; surface glabrous, innately streaked, becoming hygrophanous, viscid to glutinous, gluten colorless, margin even, rounded or slightly gibbous in outline, splitting in age, striate to translucent striate, sometimes faintly so, not becoming wrinkled or corrugate, sometimes with an edge of sterile overhanging tissue; color usually darker centrally and paler toward margin, centrally colored some shade of brown (Snuff Brown, Dresden Brown, Buckthorn Brown, Cinnamon Brown, Saccardo's Umber) to dark brown (Prout's Brown, Mummy Brown, Sepia, Bister, caput mortuum (8F7), 10YR 2/2), or dark yellow-brown, area around disc colored light orange, orange (Ochraceous Orange), brownish yellow orange (Clay Color), amber yellow-brown (Yellow Ocher, Honey Yellow), dingy yellow-brown (Old Gold, Chamois, Honey Yellow), grayish yellow, olive brown, or in age pale dark brown (Light Brownish Olive, Dresden Brown), 10YR 4/4 (dark yellowish brown), rarely Light Buff or Warm Buff, towards margin dull yellow (Light Buff, Ochraceous Buff), golden yellow (Yellow Ocher, 10YR 6/6), grayish yellow, pale grayish olive yellow (yellowish Ecru Olive, pale Olive Lake), light olive-brown (Old Gold, Isabella, dark Olive Buff) or light brown (pale Buckthorn Brown), along edge of margin yellowish white (Cartridge Buff), pale yellowish orange (pale Ochraceous Buff), grayish yellow (Antimony Yellow), 7.5YR 7/8 (reddish yellow), occasionally slightly olivaceous. Rarely concolorous as light yellow brown (Chamois), light orange (Light Ochraceous Buff), or orange-brown (Clay Color, Tawny Olive) overall, sometimes with areas of golden grayish yellow, pale olive-brown (Isabella), or innately streaked with olive brown, occasionally concolorous excluding center as amber yellow-brown (Yellow Ocher, Honey Yellow) to dark grayish yellow, olive-brown (Old Gold, dark Isabella), brownish orange, titian red, (7D6), 2.5YR 4/4 (reddish brown) or 5Y 4/4-6 (yellowish red). *Flesh* 2-5(-7) mm over lamellae at stipe apex, tapering abruptly towards margin, solid, firm, often becoming watersoaked, beneath disc colored yellowish white or darker and yellow-brown below cuticle, overall colored pale cream to yellowish white, (Light Buff, Ivory Yellow), 5Y 8/3 (pale yellow),

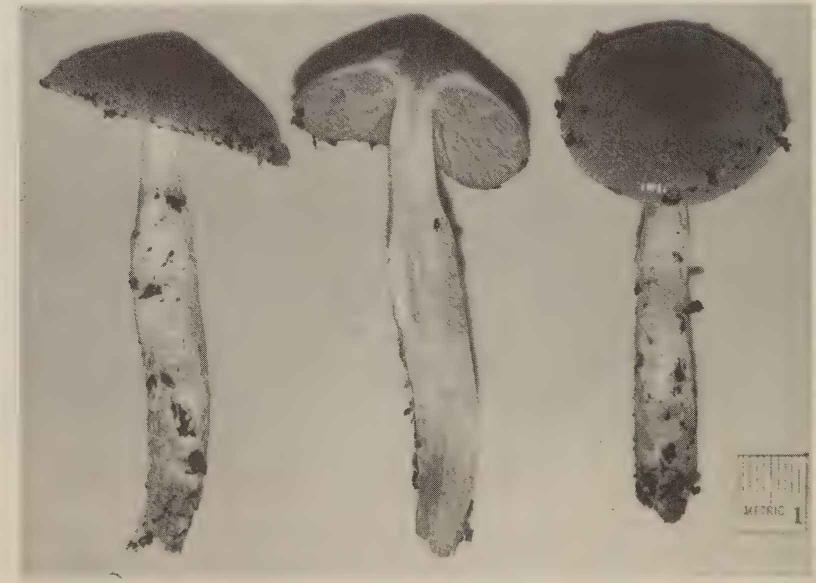

**Fig. 5.** Habit of *Cortinarius vernicosus*, MTS4346.

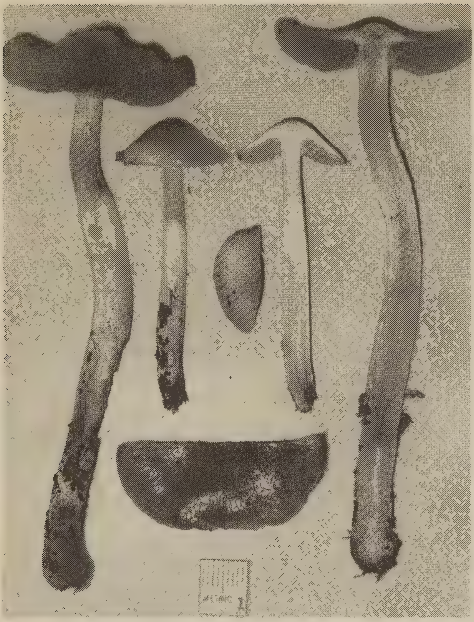

**Fig. 6.** Habit of *Cortinarius vernicosus*, MTS4341.

Straw Yellow, 5Y 8/6 (yellow), or pallid when young, becoming pale to light brown, grayish yellow, or pale gray brown in age; odor not distinctive; taste of context and gluten mild. *Lamellae* 3-6 mm wide, subdistant to close or occasionally crowded, adnate to adnexed with a decurrent line (uncinate) or slightly subdecurrent, sometimes depressed at stipe (sinuate), eventually seceding, subventricose, generally 2-4 lamellulae in two tiers, occasionally forked, (10-)12-26 lamellae + lamellulae/cm at stipe, (11-)14-24 lamellae + lamellulae/cm at pileus edge; color when young pale pinkish, pale grayish, violet white, bluish gray or pale violet (Pinkish Buff, 7.5YR 7/4 (pink), Violet Gray, Lavender), becoming grayish yellow (Chamois), golden grayish yellow (Tawny Olive), grayish orange, brownish orange (Ochraceous Tawny, pale Clay Color), and eventually dark orange-brown (Clay Color) to deep brown, edges when young slightly paler to whitish or pale violaceous-bluish, bluish gray, violet white (Pale Quaker Drab) or violet gray (17A2), in age concolorous, even or subcrenulate, becoming eroded in age, faces smooth. *Spore print* on white paper colored dark yellow-brown (Snuff Brown). *Stipe* 27-117 mm long, 1.5-8.0 mm diam at apex, 3-10 mm at widest part, equal, cylindric to subfusoid, base rounded to truncate, sometimes rooting, firm, surface smooth to slightly roughened or appressed fibrillose, apex white to pallid, dry, generally striate especially when young, streaked striate or becoming so, and occasionally scattered fibrillose, partial veil (cortina) white, sometimes leaving a subapical fibrillose zone, inner veil whitish, sometimes with violaceous or bluish tones on the surface, thin or occasionally well-developed and appearing peronate, sometimes breaking into irregular patches but never forming bands, outer surface viscid to glutinous, gluten colorless, stipe surface beneath inner veil colored whitish (Light Buff), yellowish white, pale cream to tan (Warm Buff), grayish yellow (Honey Yellow) or pale orange shades, at base colored yellow-brown (Ochraceous, Clay Color) or brownish orange (Ochraceous Tawny), where handled yellow-brown, dark brown (Mummy Brown), or brownish gray (Drab), 10YR 5/2 (grayish brown), in age becoming watersoaked, base sometimes with whitish mycelium. *Stipe interior* stuffed becoming hollow, white to cream (Light Ochraceous Buff), pale yellow, pale orange yellow, pale brown (pale Cinnamon Buff), near 10YR 6/4 (light yellowish brown) and 7.5YR 6/6 (reddish yellow), pale grayish orange, brownish yellow orange (Clay Color), yellow brown (Honey Yellow), base colored light yellow-brown to brownish, discoloring brownish orange (Ochraceous Tawny), 7.5YR 5/8 (strong brown), dark yellow-brown to yellowish brown, occasionally dark brown (dark Bister, Saccardo's Umber), yellowish brown to darker around larval tunnels.

*Chemical reactions*: KOH (3%) negative or brown on surface and context of pileus and stipe, brown on lamellae, (dark brown with 10% KOH), Melzer's reagent negative or red brown to dark brown on surface and context of pileus and stipe.

*Basidiospores* 9.4-14.1(-15.9) x 5.9-8.0 µm, $Q$=1.7-2.0, (average $Q$ = 1.9), verruculose, in face view ovate, in profile inequilateral, amygdaliform to limoniform, apiculus prominent, yellow-brown to pale orange-brown (Ochraceous Tawny) in 3% KOH, orange-brown in Melzer's reagent. *Basidia* 33-57 µm long, 9.4-15.0 µm at apex, broadly clavate, contents yellow and granular in Melzer's reagent, 4-sterigmate, rarely 2, base with clamp connection. *Hymenial cystidia* none except lamellar edge composed of occasional basidiole-like cells. *Hymenophoral trama* hyphae subparallel, 3.8-7.0(-16.5) µm diam., pale yellow ochraceous; oleiferous hyphae 2.5-7.0 µm diam. *Pileipellis* three-layered, suprapellis 131-474 µm wide, an ixotrichodermium of more or less erect intertwined hyphae in a gelatinous matrix, hyphae cylindrical, 1.9-2.8 (-5.0) µm diam., hyphal ends slightly inflated, arising from a yellow pigmented mediopellis of cylindric, interwoven, radially oriented hyphae, 9.4-23.5 x 16.9-47.0 µm, 3-5 cells thick, subpellis layer orange near mediopellis, orange-brown near pileus trama, composed of cylindric, densely interwoven, radially oriented hyphae. *Pileus trama* hyphae cylindric to fusoid, interwoven, pale orange-brown near subpellis, pale yellow to pale yellow-orange elsewhere. *Clamp connections* present throughout sporocarp.

*Habit, habitat, distribution*: solitary to scattered, terrestrial often growing in deep moss or areas of thick conifer duff, old growth mixed conifer woods of *Larix occidentalis* Nutt., *Pseudotsuga menziesii, Picea engelmanii* Parry, with scattered *Abies lasiocarpa* (Hook.) Nutt. and *Pinus contorta* Dougl. in northeastern Washington locales at 1450-1500 m (4600-4800 ft) elevation, and associated with *Pseudotsuga menziesii* and *Tsuga heterophylla* in northwestern locales, at elevations 150-315 m (500-100 ft). Sometimes fruiting in areas with very little understory, occasionally growing with *Gaultheria shallon* Pursh and/or *Vaccinium*, and *Polystichum munitum* (Kaulf.) Presl. Collected from April to early July (Fig. 2).

*Etymology:* "vernicosus", full of spring (in reference to the occurrence of sporocarps during the spring season); also varnished, shiny (in reference to appearance of glutinous veil on sporocarps when ambient conditions are dry, as commonly encountered in spring).

*Specimens examined:* USA, WA: *Ferry Co.*, Sherman Pass area, Colville National Forest, 48° 36' 27'' N, 118° 28' 42" W, 23 June 1997, *MTS4374-4376*; 24 June 1997, *MTS4384-4389*; 1 July 1995, *MTS3702* (HOLOTYPE), and *MTS3703* (PARATYPE: NYBG), *MTS3704-3705*; *Clallam Co.*, Olympic National Park, Soleduc Falls trail, 47° 57' 06" N, 123° 49' 10" W, 17 May 1994, *MTS3880* (*=TOD2161*); 5 June 1993, *MTS3653-3654*; North Fork trail, 48° 00' 23" N, 123° 49' 10" W, 17 May 1994, *MTS3879*(*=TOD2166*); Deer Creek, 47° 59' N, 123° 39' W, 12 May 1993, *TOD1698*; Deer Park, 47° 56' N, 123° 15' W, 16 June 1994, *MTS3881*; 9 June 1993, *MTS3652, MTS3655-3656*; 26 May 1993, *TOD1719* Whiskey Bend, 47° 58' 07" N, 123° 34' 54" W, 28 May 1996, *MTS4277(=TOD3457)*; Elwha River Valley, 48° 01' 41" N, 123° 35' 11" W, 7 May 1997, *MTS4345-4346, MTS4348*; 30 April 1997, *MTS4338-4342*; 19 May 1996, *MTS4274*; 11 April 1996, *MTS4270*; 2 May 1995, *MTS4042-4043, TOD2708*; 20 April 1995, *MTS4040 & 4041*; 22 April 1994, *MTS3877* (*=TOD2149*).

## Discussion

*Cortinarius vernicosus* is characterized as follows: pileus glutinous, ochraceous to orange-brown, sometimes with an olivaceous yellow brown tone mixed in; stipe equal to subfusoid, glutinous, white, rarely tinged bluish or violet; lamellae violaceous when young; spores verrucose, from 9-14 x 6-8 µm, and occurring with conifers. It is similar to *C. pumilus* (Fr.) Lange, but differs by much narrower spores, fruiting pattern in the spring, and violaceous lamellae when young. *Cortinarius trivialis* also occurs in the spring, but grows in this region strictly with *Populus*, does not have violaceous coloration, and usually develops distinctive bands on the stipe surface from separation of the whitish inner veil.

Violaceous colors may not be persistent or present on some specimens of *C. vernicosus*. Usually the young lamellae are some shade of bluish, lilac or violaceous, but these colors fade in older specimens. Also violaceous pigmentation on the stipe surface was inconsistently observed in mature fruiting bodies and is more prevalent in young specimens.

Of the 38 collections included in this study, one collection (MTS 4277) had a more yellowish pileus surface. An additional collection (MTS 4042), consisting of one very old sporocarp, had considerably larger spores (up to 19 µm). This specimen was excluded from material examined due to poor quality of the material.

Studies by Bendiksen et al. (1993) showed a significant difference in spore ornamentation using the SEM at the sectional level in subgenus *Myxacium*. Seidl (1999) found that spores in section *Defibulati* have more sharply delineated ornamentation, than those in section *Myxacium* with more rounded ornamentation. *Cortinarius vernicosus* has the more rounded, less sharply delineated type of ornamentation (Figs. 3-4), as is representative of section *Myxacium*.

The collections of *C. vernicosus* from ONP are from the Olympic Douglas-fir zone (Cassidy 1997a), occurring in mixed *Pseudotsuga menziesii* and *Tsuga heterophylla* forests. This is the driest mid-elevation zone west of the Cascade Mountain Range in Washington. In northeastern Washington near Sherman Pass, the major tree species are *Pseudotsuga menziesii, Larix occidentalis* and *Picea engelmanii*. This is considered a transition zone between the Interior Douglas-fir Forest Zone and the Subalpine Fir Zone (Cassidy, 1997b). Both areas are succeeded by the Subalpine Fir zone at higher elevations.

The geology according to Alt and Hyndman (1984), is similar in age (Eocene) for the two areas, yet the ONP localities are ocean-floor basalt and oceanic sedimentary rocks, while the eastern locality consists of granite, andesite and rhyolite. Interestingly enough this eastern area also has scattered sedimentary rocks from an ancient coastal plain that formed along the west coast of North America, where the North American Plate collided with the Pacific Ocean floor. The two localities are separated by the Cascade Mountain Range and a distance of approximately 393 km (246 mi).

According to Chapin (1968), the soils of the two areas are acidic, sandy loam to silt loam soils formed in volcanic ash, areas formed from alpine glacial materials, and scattered areas of limestone, granite, shale, and gneiss. The western ONP localities are shallow to deep, a mixture of sand, silt, gravel, and clay, with annual precipitation averaging 100 cm (40 inches). Soils of the eastern Sherman Pass area are weathered andesite, basalt, argillite, with an average annual precipitation of 140-381 cm (56-152 inches).

Regarding phenology, the slightly later fruiting mentioned for eastern Washington collections, ranged from June to July (Fig. 2). The western collections were found predominantly in April and May, with overlap in June (Fig. 2) for both areas. This difference is likely due to cooler soil temperatures in the Sherman Pass area because of the higher elevation and heavier snowpack.

In summary, six species in section *Myxacium*, including *C. vernicosus*, are known to occur in western North America. These include: *Cortinarius absarokensis* Moser & McKnight, *C. favrei* Moser ex Henderson, *C. mucosus* (Bull. ex Fr.) Kickx, *C. muscigenus* Peck, and *C. trivialis* J. E. Lange. Molecular data places *C. vernicosus* nested within section *Myxacium* with high bootstrap support (100%) using nuclear ribosomal DNA (Seidl 1999). Morphological, microscopical and phylogenetic data all support placement of *C. vernicosus* into section *Myxacium*. It is likely that this taxon occurs in other mature to old growth forests of Western North America, but possibly due to its occurrence in the spring, has thus far been overlooked.

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# CARRISMYCES, A NEW GENUS OF HYPHOMYCETES FROM A CLOUD FOREST IN MEXICO

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## ABSTRACT

***Carrismyces proliferatus*** anam. gen. & sp. nov., a new genus of hyphomycetes collected on the rotten stem of a tree fern (*Cyathea* sp.) in a Mexican cloud forest, is described and illustrated. The genus is characterized by monotretic, percurrent conidiogenous cells and obconical, subglobose, broadly pyriform to turbinate, muriform, dark brown or black conidia often with short, colourless, conical appendages. Notes on closely related genera are given.
**Keywords**: *Carrismyces*, Hyphomycetes, Mexico, systematics, tropical fungi.

## TAXONOMIC PART

***Carrismyces*** R. F. Castañeda et Heredia *anam. gen. nov.*
Etymol.: Latin *Carris*- named in honor to the American mycologist Lori Carris; -*myces* from Greek meaning fungus.

*Ad fungos conidiales, hyphomycetes pertinens.* ***Coloniae*** *in substrato naturali effusae, atrobrunnea vel nigrae.* ***Mycelium*** *partim superficiale et partim in substratum immersum.* ***Conidiophora*** *conspicua, mononematosa vel fasciculata, septata, plerumque simplicia, raro ramosa, levia vel verrucosa, brunnea vel nigra, cum hyphis adventitiis in conidiophoris et circa proliferationibus percurrentibus interdum visae.* ***Cellulae conidiogenae*** *monotreticae, integratae, percurrentes, indeterminatae, laeves vel verrucosae, brunneae vel atrobrunneae, loci conidiogeni pori apicali cum parietibus incrassantibus circa poris.* ***Secedentia conidiorum*** *schizolytica.* ***Conidia*** *obconica, subglobosa, pyriformia, turbinata usque crassa clavata, muriformia (dictyoseptata), solitaria, acrogena, levia vel verrucosa, brunnea vel nigra, interdum cum appendicibus corniformibus, hyalinis vel subhyalinis, praedita. Teleomorphosis ignota.*
*Species typica:* ***Carrismyces proliferatus*** *anam. sp. nov.*

Conidial fungi, hyphomycetes. **Colonies** on the natural substratum effuse, dark brown or

black. **Mycelium** superficial and immersed. **Conidiophores** conspicuous, mononematous or fasciculate, septate, mostly simple, rarely branched, smooth or verrucose, brown to black, with adventitious hyphae sometimes observed near percurrent proliferations. **Conidiogenous cells** monotretic, integrated, extending percurrently, indeterminate, smooth or verrucose, brown to dark brown, conidiogenous locus an apical pore, with thickened wall around the pore. **Conidial secession** schizolytic. **Conidia** obconical, subglobose, pyriform, turbinate to broadly clavate, muriform (dictyoseptate), solitary, acrogenous, smooth or verrucose, brown to black, sometimes with several corniform or conical, hyaline or subhyaline appendages.
Teleomorph: unknown.
Type species: ***Carrismyces proliferatus*** *anam. sp. nov.*

*Carrismyces* resembles *Hughesinia* J. C. Lindq & Gamundí (Ellis, 1976) in its conidium ontogeny: in both genera the conidiogenous cells are monotretic and the conidia are solitary, acrogenous, and brown to dark brown, but there are clear differences in the development of conidial septa and appendages. The mature conidia of *Hughesinia* have 2-4 columns of cells which diverge from a common basal cell and seem to develop independently, each column ending in an apical tapered appendage like that in *Tetraploa* (Ellis, 1976). The mature conidia of *Carrismyces* are typically dictyoseptate. The first septum divides the conidium into two hemispherical portions, then septation continues randomly. In addition, occasional lobed hyphopodia are present on the vegetative hyphae of *Hughesinia*, but only adventitious hyphae are observed in *Carrismyces*. The adventitious hyphae of *Carrismyces* develop as one or several hyphal structures arising from the base of conidiogenous cells after each percurrent proliferation and growing towards the base, sometimes anastomosing close to the percurrent proliferation and forming a rudimentary stroma. The 1-5 corniform or conical appendages in *Carrismyces* arise from different cells around the periphery of the conidium wall and not at the end of columns as in *Hughesinia*.

*Tretospeira* Piroz. (Pirozynski, 1972) also resembles *Carrismyces*, but the former has conidia made of 4 or more parallel columns of cells, like *Dictyosporium* Corda as pointed out by Pirozynski (1972). Other genera similar in conidium ontogeny and conidial shape to *Carrismyces* are *Piricauda* Bubák (Ellis, 1971, 1976) and *Piricaudiopsis* J. Mena & Mercado (Mena-Portales and Mercado-Sierra, 1987; Bhat and Kendrick, 1993). In *Piricauda* the conidiophores are not differentiated and the determinate conidiogenous cells lack the percurrent proliferations seen in *Carrismyces*.

*Piricaudiopsis* has discrete, integrated and determinate conidiogenous cells and the dictyoseptate conidia are variable in shape with several long, septate, divergent, brown appendages. *Piricaudiopsis* is thus clearly differentiated from *Carrismyces*. *Piricaudilium* Hol.-Jech. (Holubová-Jechová, 1988) which is very close if not identical to *Piricauda*, also has undifferentiated conidiophores and determinate conidiogenous cells. Some described species of *Acrodictys* M. B. Ellis (Ellis, 1971, 1976) resemble *Carrismyces* in their conidial morphology, but their conidium ontogeny is quite different.

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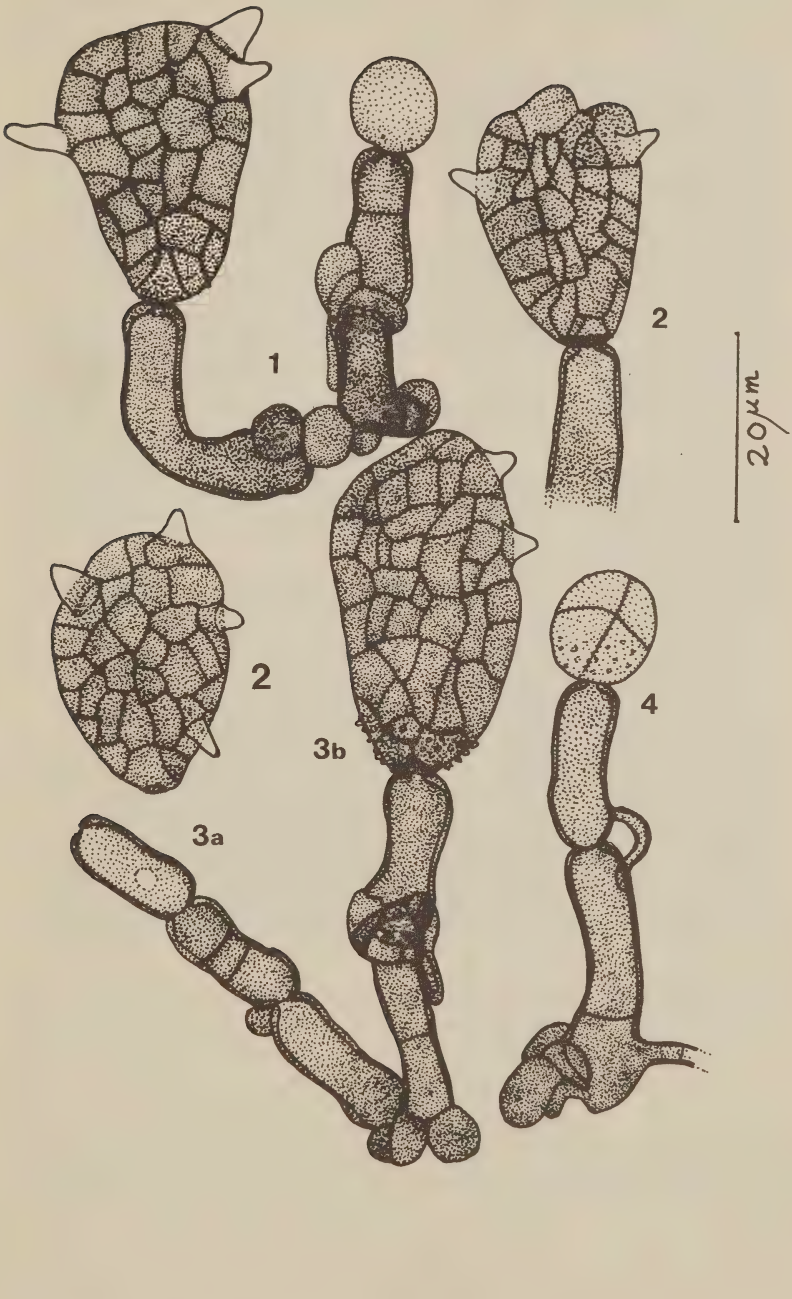
**Figs. 1-4.** ***Carrismyces proliferatus***. **1**. Fasciculate conidiophores showing adventitious hyphae and attached conidia. **2.** Obconical conidia with corniform appendages. **3a.** Conidiogenous cell. **3b.** Mature conidium with verrucose base. **4.** Developing conidium on conidiophore.


1
2
20µm
2
3b
4
3a

The genera *Bioconiosporium* Batista & Bezerra (Ellis, 1976 ), *Oncopodiella* Arnaud: Rifai (Ellis, 1971), *Pseudopetrakia* M. B. Ellis (Ellis, 1971a, 1976) and *Septosporium* Corda (Ellis, 1971) are somewhat similar to *Carrismyces* in their dictyoseptate conidia, but *Bioconiosporium* and *Oncopodiella* have inconspicuous conidiophores and monoblastic and polyblastic, determinate conidiogenous cells.

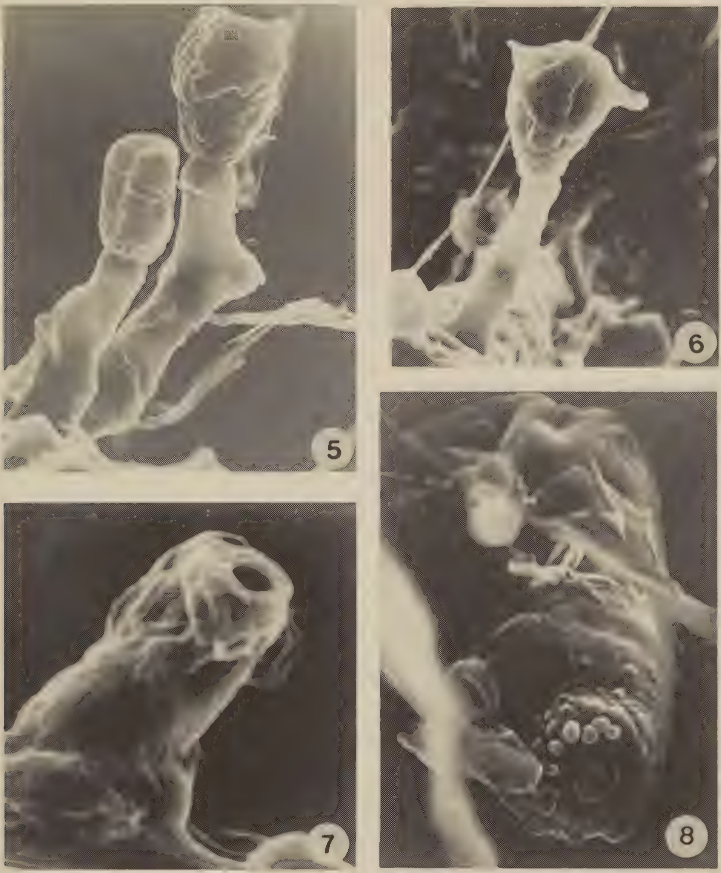
*Pseudopetrakia* also is characterized by monoblastic, determinate conidiogenous cells. In *Septosporium*, numerous simple, subulate, dark brown to black, thick-walled setae arise from vegetative hyphae, and the conidiogenous cells also are monoblastic; moreover, one or more cells of the conidium develop into a pycnidial synanamorph.

***Carrismyces proliferatus*** R. F. Castañeda et Heredia *anam. sp. nov.* Figs. 1-8.
Etymol.: Latin *proliferatus*- referring to the growth of the conidiogenous cells.

***Coloniae** in substrato naturali effusae, pilosae, mycophilae vel lignatiliae, atrobrunnea vel nigrae. Mycelium partim superficiale et partim in substratum immersum. Hyphae septatae, ramosae, leves, brunneae vel atrobrunneae, 2.0-3.5* µm *diam.* ***Conidiophora*** *conspicua, mononemata, interdum 2-3 aggregata, septata, erecta, recta vel flexuosa, levia, brunnea vel nigra ad basim, brunnea ad apicem, ad septa constricta, 10-70 x 6-10* µm, *plerumque simplicia, raro ramosa, (sed circa basim unusquisque cellulis conidiogenis 1-4-hyphis adventitiis, 4-8* µm *latis, inflatis, lateraliter descendentibus, interdum anastomosantibus).* ***Cellulae conidiogenae*** *monotreticae, cylindricae usque subclavatae vel subdoliiformes, integratae, percurrentes, indeterminatae, laeves, brunneae vel atrobrunneae, 10-20 x 6-7* µm. ***Loci conidiogeni*** *pori apicali.* ***Secedentia conidiorum*** *schizolytica.* ***Conidia*** *obconica, subglobosa, pyriformia vel turbinata, muriformia (dictyoseptata), solitaria, acrogena, 23-40 x 17-23* µm, *levia, raro verrucosa prope basim, brunnea vel nigra, interdum cum 1-5- appendicibus corniformibus vel conicis obtusis ad apicibus, laevibus, hyalinis vel subhyalinis, aseptatis, 4-8 (-12) x 3.0-4.5* µm, *praedita. Teleomorphosis ignota.*
**Matrix:** *In rami emuortis putridis Cyatheae sp. et setis fungus non determinato.*
**Habitat:** *In sylva pluvialis "Las Cañadas", 6 Km usque ad Huatusco, Veracruz, Mexico, 1300 m altitude. Legit. R. F. Castañeda et G. Heredia. 20. VII. 1999. Holotypus: CB736 XAL, Xalapa, Mexico. Isotypus: MUCL 41731.*

**Colonies** on the natural substratum effuse, hairy, mycophilous and lignicolous, dark brown to black. **Mycelium** superficial and immersed hyphae septate, branched, smooth, brown to dark brown, 2.0-3.5 µm diam. **Conidiophores** conspicuous, mononematous, sometimes 2-3 in a group, septate, erect, straight or flexuous, smooth, brown to black at the base, brown towards the apex, 10-70 x 6-10 µm, singly, rarely branched, but near each proliferation 1-4 adventitious hyphae 4-8 µm wide, arise from the base of the conidiogenous cells after each percurrent proliferation and grow downward close to the wall of the conidiophores. **Conidiogenous cells** monotretic, cylindrical to subclavate or doliiform, integrated, indeterminate, smooth, brown to dark brown, constricted at septa, 10-20 x 6-7 µm. **Conidiogenous locus** an apical pore. **Conidial secession** schizolytic. **Conidia** obconical, subglobose, pyriform to turbinate, muriform (dictyoseptate), solitary, acrogenous, 23-40 x 17-23 µm, smooth, sometimes verrucose near the base, brown or black, sometimes with 1-5 short, corniform or conical appendages, obtuse at the apex, hyaline or subhyaline, smooth, unicellular, 4-8 (-12) x 3.0-4.5 µm. Teleomorph: unknown.

**Matrix:** On rotten branch of *Cyathea* sp. and on setae of unidentified fungus. Habitat: In a cloud forest, "Las Cañadas", 6 Km from Huatusco, Veracruz, Mexico, 1300 m altitude. Col. R. F. Castañeda et G. Heredia 20. VII. 1999. Holotype: CB736 XAL, Xalapa, Mexico. Isotype: MUCL 41731.



**Figs. 5-8.** ***Carrismyces proliferatus*** 5. Conidiophores with conidia attached (2000x). **6.** Mature conidia with appendages (1500x). **7.** Conidiogenous cell showing apical pore. **8.** Conidium (5000x).

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MYCOTAXON

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# A NEW SPECIES OF NETA FROM LEAF LITTER IN MEXICO

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## ABSTRACT

*Neta gigaspora* anam. sp. nov. is described and illustrated from rotten leaves collected in the rain forest of Chajul, Chiapas, Mexico. It is characterized by cylindrical, 5-7-septate, guttulate conidia arising from long, denticulate conidiogenous cells. Notes on closely related species and a key to *Neta* species are provided.

**Keywords**: *Neta*, Hyphomycetes, systematics, tropical fungi, Mexico.

## TAXONOMIC PART

The genus *Neta* Shearer & J. L. Crane (1971) includes species with hyaline, subhyaline, conspicuous, septate, simple or branched conidiophores which bear sympodially proliferated, integrated, indeterminate, hologenous, multilocal conidiogenous cells with long protuberant conidiogenous loci (denticles), and exhibit schizolytic conidial secession. The conidia are solitary, reniform, cylindrical, sublunate to allantoid, dumbbell-shaped or gibbous, 0- to several-septate, hyaline, and smooth. *Neta* includes nine terrestrial or aquatic species: *N. patuxentica* Shearer & J. L. Crane (1971, the type species), *N. lignicola* Shearer (1974), the *Neta* synanamorph of *Cladosporium bisporum* Matsush. (1975), *N. quadriguttata* (Matsush.) de Hoog (1985), the *Neta* synanamorph of *Ramichloridium ovoideum* (Matsush.) de Hoog, in Matsushima (1985), *N. compacta* de Hoog (1985), *N. salmonicolor* (Shearer) de Hoog (1985), *N. gibbosospora* R. F. Castañeda & W. B. Kendr. (1990) and *N. angliae* K. D. Hyde & Goh (1999). A new species is proposed below.

***Neta gigaspora*** R. F. Castañeda & Heredia anam. sp. nov.
Etymol.: from Greek *giga-* (very large) and *-spora* (spore).

*Ad fungus conidiales, hyphomycetes pertinens. Coloniae in substrato naturali effusae, plumosae usque ad caespitosae, hypophyllae, albae vel ochraceae. Mycelium plerumque in substrato immersum, ex hyphis septatis, ramosis, laevibus, hyalinis, 1.0-1.5 μm diam. compositum. Conidiophora conspicua or undifferentiated, mononemata, cylindrica,* *leviter inflata ad basim, erecta, recta vel flexuosa, simplicia, raro ramosa, 0-1-septata, 20-35 x 5-6 µm, levia, hyalina, interdum in cellula conidiogena reducta. Cellulae conidiogenae multiloculatae, hologenae, sympodiales, indeterminatae, hyalinae, in conidiophoris incorporatae. Conidiogeni loci longi denticulati, 2-4 µm longi. Conidiorum secessio schizolytica. Conidia cylindrica, rotundata ad apicem, leviter curvata ad basim, solitaria, 5 (-7)-septata, levia, 32-42 x 4-5 µm, guttulata, hyalina. Teleomorphosis ignota.*

Matrix: In foliis dejectis putridis non determinatae. Habitat in sylva pluvialis Chajul, Chiapas, Mexico. Holotypus: R. Guevara, 10 VI 1999. XAL 737, Xalapa, Mexico.

Conidial fungi, hyphomycetes. **Colonies** on natural substratum effuse, feathery to caespitose, hypophyllous, white or ochraceous. **Mycelium** mostly immersed, composed of 1.0-1.5 µm diam., septate, branched, smooth, hyaline hyphae. **Conidiophores** 20-35 x 5-6 µm, conspicuous, mononematous, cylindrical, slightly inflated at the base, erect, straight or flexuous, simple or rarely branched, 0-1-septate, smooth, hyaline, sometimes reduced to conidiogenous cells. **Conidiogenous cells** multilocal, hologenous, sympodially proliferating, indeterminate, hyaline, integrated. Conidiogenous loci 2-4 µm long, protuberant, with several distinct denticles. Conidial secession schizolytic. **Conidia** 32-42 x 4-5 µm, cylindrical, rounded at the apex, gently curved towards the base, solitary, 5 (-7)-septate, smooth-walled, guttulate, hyaline. Teleomorph unknown.

Matrix: On fallen rotten leaves of an unidentified plant. Habitat: Rain forest of Chajul, Chiapas, Mexico. Holotype: R. Guevara, 10 VI 1999. XAL 737, Xalapa, Mexico.

Among previously described species, only *N. quadriguttata* resembles *N. gigaspora* in conidial shape, but the former has 3-septate conidia measuring 18-30 x 5-7 µm, while *N. gigaspora* has 5 (-7)-septate conidia which measure 32-42 x 4-5 µm. Matsushima (1985) remarks that the synanamorph of *Ramichloridium ovoideum* (Matsush.) de Hoog (1977) is similar to *Neta quadriguttata* (as "*Dactylaria quadriguttata*"). Comparing the descriptions and illustrations of *Cladosporium bisporum* and *Ramichloridium ovoideum* given by Matsushima in 1975 and 1985, respectively, both species are close, if not identical, as pointed out de Hoog (1985). *Ramichloridium ovoideum* has denticulate conidiogenous cells that are different from those of the type species, *R. apiculatum* (Miller, Giddens & Foster) de Hoog (1977), and could be accommodated in either *Neta* or *Dactylaria*, but a new combination is not proposed at this time.

## KEY TO THE ACCEPTED *NETA* SPECIES

1 . Conidia unicellular ........................................ 2
1'. Conidia septate ........................................ 3

2 . Conidia suballantoid, guttulate, smooth, hyaline, curved, 7-10 x 2-3 µm ........................................ ***N. lignicola***
2'. Conidia cylindrical, gibbous, slightly truncate at the base, rounded at the apex,hyaline, smooth, 4-7 x 1.5-2.5 µm .................................. ***N. gibbosospora***

3 . Conidia 1-septate ........................................ 4
3'. Conidia with more than 1 septum ........................................ 6

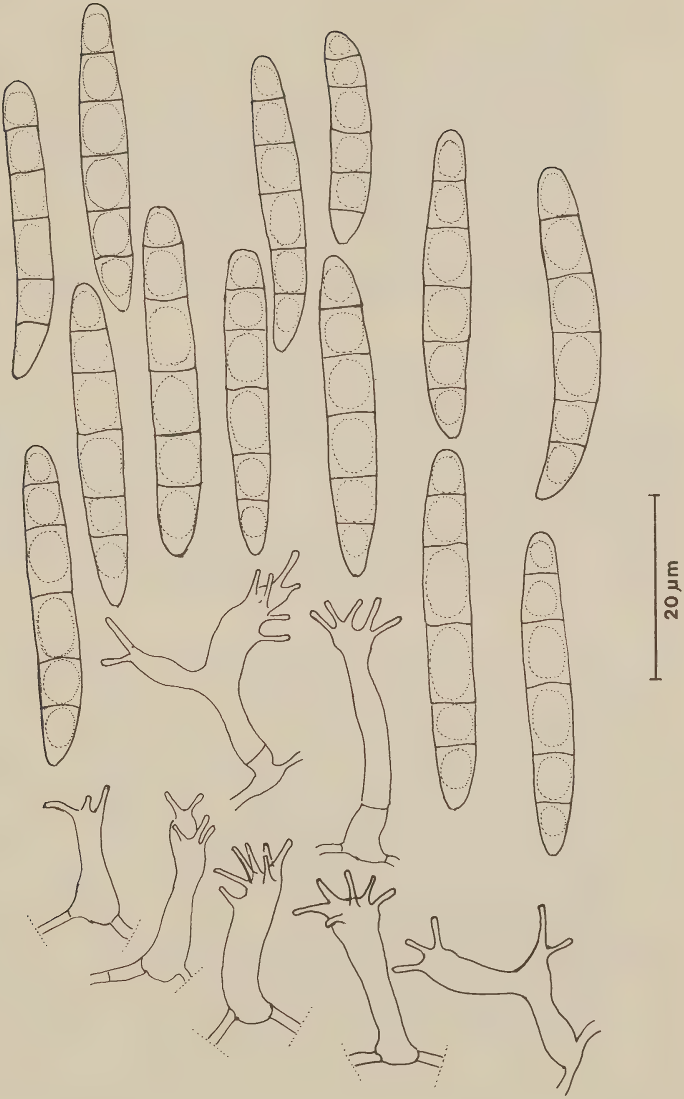

Fig. 1. *Neta gigaspora*. Conidiophores and conidia

4 . Conidia dumbbell-shaped, strongly constricted at septum, hyaline, smooth, 9-13 x 3-4 µm ........ ***N. compacta***
4'. Conidia not dumbbell-shaped ........ 5

5 . Conidia reniform to allantoid, hyaline, smooth, 11-15 x 3.0-4.5 µm ........ ***N. patuxentica***
5'. Conidia ellipsoid, obovoid, sometimes slightly curved, guttulate, 9-12 x 3.5-4.5 µm ........ ***N. salmonicolor***
5''. Conidia oblong-ellipsoidal or suballantoid, thin-walled, smooth, hyaline, 10-13 x 2-3 µm ........ ***N. angliae***

6 . Conidia 3-septate, cylindrical, curved, guttulate, hyaline, smooth, 17-30 x 5-7 µm ........ ***N. quadriguttata***
6'. Conidia 5 (-7)-septate, cylindrical, rounded at the apex, slightly curved, smooth, guttulate, hyaline, 32-42 x 4-5 µm ........ ***N. gigaspora***

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# BOTRYOSPHAERIA POPULI SP. NOV. AND ITS FUSICOCCUM ANAMORPH FROM POPLAR TREES IN PORTUGAL

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## ABSTRACT

A species of *Botryosphaeria*, *B. populi*, and its anamorph, *Fusicoccum populi* are newly described from poplar trees in Portugal. *Botryosphaeria populi* differs from other species in the genus by its long, narrow asci and the long, paraphyses originating from the layer of cells giving rise to conidiophores and conidiogenous cells of its anamorph. Only one other *Fusicoccum* sp. has been described with paraphyses and it is distinctly different from *F. populi*.

**Keywords:** *Botryosphaeria, Fusicoccum*, paraphyses, *Populus nigra*

## INTRODUCTION

Following Sivanesan's (1984) treatment of 12 species of *Botryosphaeria* Ces. & De Not., several additional species have been described (Pennycook & Samuels, 1985; Sivanesan & Sutton, 1985; Bisset, 1986; Shang, 1987; Gardner & Hodges, 1988; Ramesh, 1991; Subileau *et al.*, 1994; Yuan, 1996; Gardner, 1997; Denman *et al.*, 1999). Because teleomorphic characters vary little between species, the taxonomy of the group is based primarily on characteristics of the anamorphs.

During a study of *Botryosphaeria* species in Portugal a fungus, initially thought to be *Botryosphaeria dothidea* (Moug.: Fr.) Ces & De Not., was found on dead branches of *Populus nigra* L. However, conidia of the anamorph were longer than normally reported for *B. dothidea* (Kobayashi & Oishi, 1979; Crous & Palm, 1999) and long paraphyses were intermingled with the conidiophores. Although more than 200 taxa have been described in *Fusicoccum* Corda (Sutton, 1980) only one other species, *F. proteae* Denman & Crous, has been reported to have such structures (Denman *et al.*, 1999). The anamorph of the species on poplar was distinctly different from the anamorph of *F. proteae* and for this reason it is described here as new.

## MATERIALS AND METHODS

Hand-cut sections were mounted in lactophenol and examined by Nomarski differential interference contrast microscopy. Single ascospore and single conidium isolates were established on Difco potato dextrose agar (PDA) and cultures were incubated at ca. 20°C with daylight from a north-facing window.

## RESULTS

***Botryosphaeria populi*** **A.J.L. Phillips, sp. nov.** (Fig. 1a, b)

Stromata per texturam hospitis erumpentia, atrobrunnea ad nigra, 0.5–1.5 mm diam., multiloculata, ostiolati, papillata, canalem ostiolarem non periphysatum. Loculi sphaerici ad ovatos, 100–160 μm diam. Pseudoparaphyses filamentosae, hyalinae, septatae, ramosae, 3–4 μm diam. Asci 140–180 × 17–24 μm, clavati, bitunicati, octospori. Ascosporae (22–)24–27(–29) × 8–10(–11) μm, aseptatae, hyalinae, parietibus tenuibus, laevia, contentis reticulate granulosis, late fusiformes ad ellipsoides.

Stromata erumpent through the bark, dark brown to black, 0.5–1.5 mm diam., multiloculate, ostiolate, papillate, ostiolar canal non-periphysate; locules spherical to ovoid, 100–160 μm diam. Ascomata formed around the periphery of and confluent with a central conidioma; cells of the stroma pseudoparenchymatous, thick-walled, dark brown becoming flattened and thinner-walled towards the inner layers. Pseudoparaphyses filamentous, hyaline, septate, branched, 3–4 μm diam. Asci forming in a hymenium layer in the lower third of the ascomatal cavity, 140–180 × 17–24 μm, clavate, bitunicate, 8-spored. Ascospores (22–)24–27(–29) × 8–10(–11) μm, aseptate, hyaline, thin-walled, smooth with granular or reticulately textured contents, broadly fusiform to ellipsoid with blunt ends.

Single ascospore and single conidium cultures gave rise to the anamorph only and this differed from other species in the genus. A new species name is therefore proposed.

***Fusicoccum populi*** **A.J.L. Phillips, sp. nov.** (Fig. 1c – e)

Conidiomata pycnidialia, eustromatica ad 300 μm diam., atrobrunnea ad nigra, uni- ad multilocularia. Conidiophorae hyalinae, laeves, subcylindricae, septatae, 23–35 × 4–5 μm, paraphysibus hyalinis, septatis inmixtae e cellulas iisdem atque conidiophoris, usque 110 × 2.5–6 μm, in conidiomatibus plerumque abundantes. Conidiogenae cellulae holoblasticae, hyalinae, laeves, cylindricae, enteroblastice et percurrenter proliferates vel phialidibus typicus periclinaliter spissescentibus. Conidia hyalina, parietibus tenuibus, aseptata, laevia, fusiformia, apice subobtuso, base truncata, (24–) 26–32(–36.5) × 5–6 μm.

Mycelium immersed, consisting of septate, branched, smooth, medium brown hyphae, 2.5–5 μm diameter. Conidiomata eustromatic, pycnidial, confluent, dark brown to black, subperidermal, locules up to 300 μm diam., walls consisting of dark brown *textura angularis*, becoming smaller and hyaline towards the conidiogenous region. Ostioles, papillate, circular. Conidiophores hyaline, smooth, thin-walled, rarely branched at the base, cylindrical, formed from the cells of the inner locule wall, 23–35 × 4–5 μm, intermingled with hyaline, septate paraphyses up to 110 μm long, 2.5–6 μm wide at base, tapering to acutely rounded apices 2–2.5 μm diam. at the tip. Conidiogenous cells discrete or integrated, hyaline, smooth, cylindrical, producing the first conidium holoblastically, and subsequent ones enteroblastically, proliferating percurrently with 2–3 indistinct percurrent proliferations, or determinate with typical phialides and periclinal thickening (*sensu* Sutton, 1980), 13–28 × 4–5 μm. Conidia hyaline, thin-walled, aseptate, smooth, fusiform, widest in the middle or upper third of

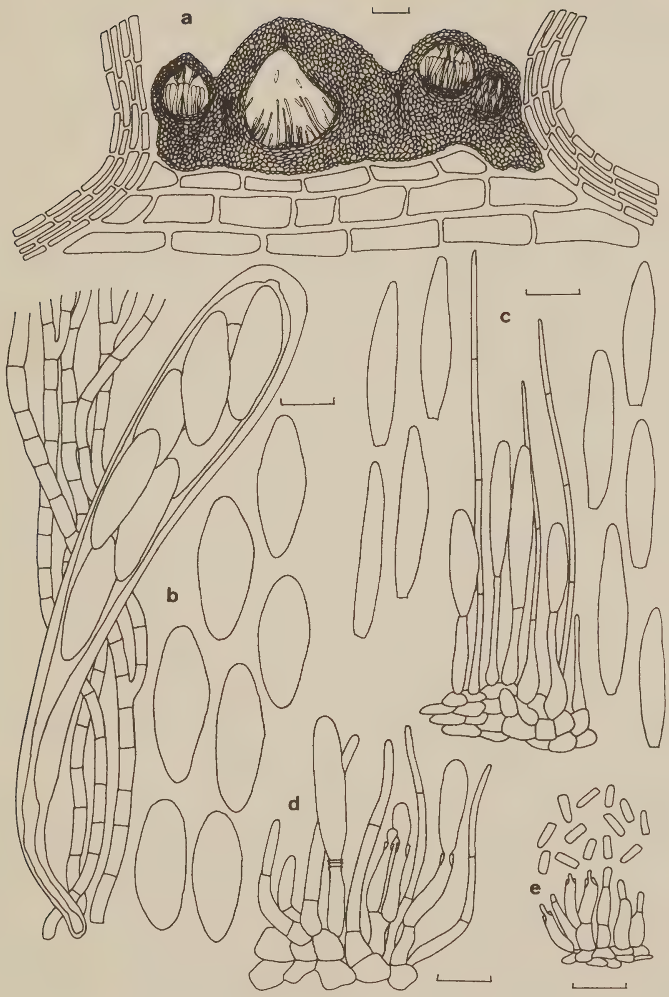

Figure 1. a & b: *Botryosphaeria populi*. a: Vertical section through a stroma; b: Ascus, ascospores and pseudoparaphyses. c – e: *Fusicoccum populi*. c: holoblastic and d: enteroblastic, phialidic conidiogenous cells, conidia and paraphyses. e: spermatiogenous cells and spermatia. Scale bars: a = 100 µm; b – e = 10 µm.

the conidium, apex subobtuse, base truncate (24–)26–32(–36.5) × 5–6 µm. Spermatiophores hyaline, smooth, occasionally branched, cylindrical, septate, 10–15 µm × 2–3.5 µm. Spermatiogenous cells discrete or integrated, hyaline, smooth, cylindrical, holoblastic or proliferating via determinate phialides with periclinal thickening, 7–10 × 2–3 µm. Spermatia hyaline, smooth, aseptate, rod-shaped with rounded ends, 4–5.5 × 2 µm.

HOLOTYPE (for both species): PORTUGAL. MINHO: Braga, São Vitor, *Populus nigra* L., 29 Dec. 1999, A.J.L. Phillips (LISE 94446, culture ex-type CAP 086).

## DISCUSSION

*Botryosphaeria* species are widely distributed on a range of woody hosts causing diebacks and cankers on stems. This genus of bitunicate ascomycetes is characterized by multi- or uni-loculate ascomata occurring separately, or grouped to aggregated on a common basal stroma (Sivanesan, 1984). Pseudothecia are ostiolate, and either embedded in the host tissue or erumpent. Ascomata and conidiomata frequently occur in the same stroma. The arrangement of ascomata around a central conidioma in the specimens examined in this work was distinctive, but this may be a result of the host or climatic factors and cannot be regarded as a stable character for species differentiation.

Von Arx & Müller (1954) cited extensive synonymy for *B. dothidea* but their work was based entirely on a study of herbarium specimens of the teleomorph and no cultural or anamorphic characters were taken into account. Because of the wide range of spore dimensions associated with the anamorph of *B. dothidea* (Kobayashi & Oishi, 1979) and from the cultural work of Pennycook & Samuels (1985) it seems that *B. dothidea* is a species complex. The fungus examined here undoubtedly belongs in the genus *Botryosphaeria* and the general characters of the teleomorph could place it within the *B. dothidea* complex. However, the asci are longer than reported for *B. dothidea* (Pennycook & Samuels, 1985) and most other hyaline-spored species of *Botryosphaeria* (Kobayashi & Oishi, 1979). Although asci fall within the range of lengths reported for *B. tsugae* Funk (Funk, 1964) and *B. mamane* Gardner (Gardner, 1997), asci of *B. populi* are much narrower than in both these species. Since the teleomorphs of the *B. dothidea* complex are morphologically indistinguishable, species are primarily differentiated on the basis of anamorph characters.

Anamorphs of *Botryosphaeria* species have been placed in the genera *Botryodiplodia* (Sacc.) Sacc., *Dothiorella* Sacc., *Diplodia* Fr., *Fusicoccum* Corda, *Lasiodiplodia* Ellis & Everh., *Macrophoma* (Sacc.) Berl. & Vogl., *Macrophomopsis* Petrak, and *Sphaeropsis* Sacc. (Sutton, 1980; Sivanesan, 1984). These genera were not clearly delimited because the morphological features that separate them were poorly defined. However, considerable progress has been made towards stabilizing species concepts. Thus, Sutton (1980) considered *Macrophoma* a synonym of *Sphaeropsis*, and Pennycook & Samuels (1985) regarded *Macrophomopsis* to be a synonym of *Fusicoccum*. Crous & Palm (1999) established that the type species of *Botryodiplodia* is a valsoid fungus and, therefore, the name cannot be used for a coelomycete. They also showed that the type specimen of *Dothiorella* is better placed in the genus *Diplodia*. Although the status of *Lasiodiplodia* has not been critically evaluated, Denman *et al.* (2000) suggest that it should be included as a synonym of *Diplodia*. Thus, of the eight genera in which anamorphs of *Botryosphaeria* spp. have been placed, only three can be considered with any degree of confidence. Of these three, the anamorphic fungus studied here clearly fits within the genus *Fusicoccum*.

The concept of *Fusicoccum* proposed by Sutton (1980) includes coelomycetes with fusiform, hyaline, non-septate conidia produced holoblastically in stromatic conidiomata. This concept was based on a Saccardo specimen from *Aesculus* in Italy because Corda's type specimen is apparently no longer extant (Sutton, 1980; Pennycook & Samuels, 1985). However, Pennycook & Samuels (1985) pointed out that the Saccardo specimen is immature and the concept should be expanded to include species with phialidic conidiogenous cells that may proliferate percurrently. Pennycook & Samuels (1985) and Phillips & Lucas (1997) further broadened the concept of *Fusicoccum* to include taxa with conidiomata ranging from unilocular pycnidia to complex eustromatic structures. Crous & Palm (1999) confirmed these observations, designated the Saccardo specimen as neotype of *F. aesculi* and provided an emended description of the genus. *Fusicoccum populi*, which has paraphyses, further broadens the generic concept of *Fusicoccum*.

The long paraphyses intermingled with the conidiophores, often extending beyond the tips of the developing conidia, is one of the major distinguishing features of *F. populi*. Paraphyses were most abundant in young conidiomata with predominantly holoblastic conidia but were also present in older ones with phialidic conidiogenesis and exuding conidia from their ostioles. Paraphyses have been mentioned rarely in coelomycetes. According to Zambettakis (1954) they are a characteristic feature of the *Lasiodiplodia* and *Diplodia* anamorphs of some *Botryosphaeria* species. Interestingly, Bender (1934) referred to paraphyses in *Macrophomopsis*, a genus which Pennycook & Samuels (1985) regarded as a synonym of *Fusicoccum*. However, in proposing the synonymy, Pennycook & Samuels (1985) did not mention these structures. Sutton (1980) defined paraphyses as sterile hyphae which are free at the apex and often produced among fertile conidiophores or conidiogenous cells. In *F. populi* paraphyses arose from the cell layers that gave rise to the conidiophores and conidiogenous cells but were narrower and their tips rounded in contrast to the broader conidiogenous cells with truncate tips. They were present early in development and persisted in older conidiomata. It seems unlikely that they become transformed into conidiogenous cells as may occur in *Phomopsis javanica* Uecker & Johnson and *P. longiparaphysata* Uecker & Kuo and (Uecker & Johnson, 1991; Uecker & Kuo, 1992). It is possible that some of the shorter, apparently sterile structures are in fact immature conidiophores. However, it is most unlikely that the longer structures will become transformed into conidiophores since such long conidiophores were never seen in any of the preparations examined, and paraphyses were found in older conidiomata in which many of the conidia were produced enteroblastically on phialides.

The only other species of *Fusicoccum* that has been described with paraphyses is *F. proteae* (Denman, *et al.*, 1999). *Fusicoccum populi* differs from *F. proteae* in having much longer paraphyses and longer conidia. Because of the presence of a *Sphaeropsis*-like synanamorph and certain features of the teleomorph, Denman *et al.* (1999) had certain reservations about whether *F. proteae* is a true *Fusicoccum*. *Fusicoccum populi*, on the other hand, undoubtedly falls within the concept of the genus *Fusicoccum*.

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# STRIATODECOSPORA GEN. NOV. FROM BAMBOO IN HONG KONG

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## ABSTRACT

From collections of fungi on bamboo in Hong Kong, we identified an interesting, new taxon. The fungus has affinities with *Spirodecospora*, but differs in that it lacks the verrucose ascospores with spiral wall ornamentation and the surrounding fibrillar sheath of *Spirodecospora*. *Striatodecospora* gen. nov. is described and illustrated to accommodate this taxon.

**Key words:** bamboo fungi, *Bambusa chungi*, *Spirodecospora*, Xylariaceae.

## INTRODUCTION

During a study of fungi on bamboo in Hong Kong we collected an *Anthostomella*-like species on dead bamboo culms in Tai Po Kau Nature Reserve, The New Territories. The collections could not be suitably placed in *Anthostomella*, or other related genera in the Xylariaceae, because the ascospores are covered with conspicuous striate ornamentation and lack germ slits or pores. *Striatodecospora* gen. nov. is therefore introduced to accommodate this taxon, with *S. bambusae* as the type of this monotypic genus.

## MATERIALS AND METHODS

Dead culms of *Bambusa chungii*, collected from Tai Po Kau Nature Reserve, The New Territories, Hong Kong, were returned to the laboratory and incubated in polythene bags lined with moistened tissue. Material was periodically examined for the presence of fungal fruiting bodies. Single spore isolations of *Striatodecospora bambusae* were attempted, but the ascospores failed to germinate. All microscopic measurements were taken from specimens mounted in water.

## DESCRIPTIONS

***Striatodecospora*** D. Q. Zhou, K. D. Hyde and B. S. Lu, gen. nov.

Ascomata immersa, obpyriformia, ostiolata, paraphysata. Asci 8-spori, cylindrici, unitunicati, ad apicem rotundati, jodi ope azurescens praediti. Ascosporae late ellipsoideae, brunneae, unicellulae, cum striatiter ornamentae.

**Ascomata** deeply immersed in host, obpyriform, ostiolate, often solitary, but sometimes in pairs or gregarious. **Peridium** comprising two layers, an inner layer of hyaline or pale brown, flattened cells, and an outer layer of dark brown, thick-walled, angular or compressed cells, fusing with host cells at the periphery. **Paraphyses** filamentous, numerous, septate, branched and longer than asci with free ends. **Asci** 8-spored, cylindrical, short-pedicellate, unitunicate, apically rounded, with a J+, wedge-shaped, subapical ring. **Ascospores** uniseriate or partly overlapping uniseriate, ellipsoidal, brown, unicellular, lacking germ slits or germ pores, with conspicuously striate ornamentation.

**Etymology:** from the Latin *striato* meaning “striate” and *deco* meaning “decoration” in reference to the striate ornamentation on the ascospores.

**Typis generis:** *Striatodecospora bambusae* D. Q. Zhou, K. D. Hyde and B. S. Lu.

***Striatodecospora bambusae*** D. Q. Zhou, K. D. Hyde and B. S. Lu, sp. nov.

(Figs. 1-12)

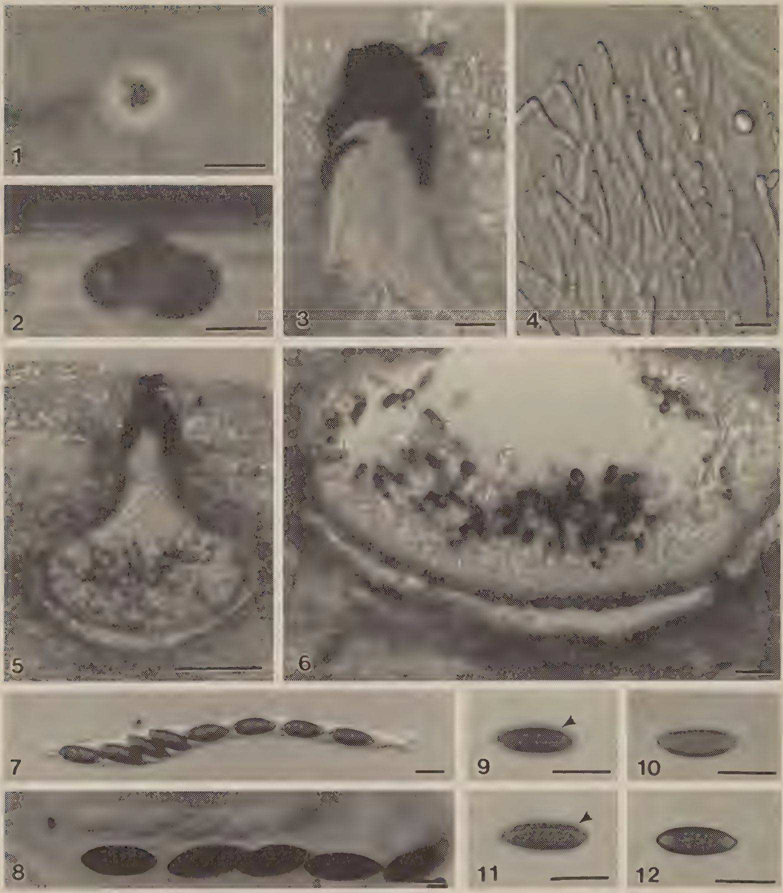
Ascomata (190-) 215-230 (-245) µm in diametro, (260-) 285-305 (-320) µm alta, obpyriformia, immersa, solitaria. Asci (110-) 118-132 × 10-12 µm, octospori, cylindrici, subapicale rotundati, cum annulis subapicalis, J+ cuneatis 1.3-2.5 × 2.5 µm, praediti. Ascosporae 14-16 × 5-6 µm, ellipsoideae, brunneae, striatiter ornamentae, fissura et poro germinatio non praeditae.

**Ascomata** deeply immersed in host, visible as blackened flattened dots, often surrounded by black cluster of ascospores, usually solitary, in vertical section (190-) 215-230 (-245) µm diam. ($\bar{x}$ = 220.5 µm, n = 10), (260-) 285-305 (-320) µm high ($\bar{x}$ = 295 µm, n = 10), obpyriform, periphysate, with a central, ostiolar canal, 60-85 µm diam., 170-275 µm high (Figs. 1-3, 5). **Peridium** 14-22 µm wide ($\bar{x}$ = 17 µm, n = 15), comprising two layers, an inner layer of hyaline or pale brown, flattened cells, and an outer layer of dark brown, thick-walled, angular or compressed cells, fusing with host cells at the periphery (Fig. 6). **Paraphyses** 2-4 µm wide, filamentous, numerous, septate, branched, longer than asci and embedded in a gelatinous matrix (Fig. 4). **Asci** (110-) 118-132 × 10-12 µm ($\bar{x}$ = 125 × 11 µm, n = 15), 8-spored, cylindrical, short-pedicellate, unitunicate, apically rounded, with a J+, wedge-shaped, subapical ring, 2.5 µm diam. ($\bar{x}$ = 2.5 µm, n = 10), 1.3-2.5 µm high ($\bar{x}$ = 2 µm, n = 10) (Figs. 7-8). **Ascospores** 14-16 × 5-6 µm ($\bar{x}$ = 14.5 × 5.5 µm, n = 30), uniseriate or sometimes partly overlapping uniseriate, ellipsoidal, brown, unicellular, containing one lipid globule, with striate ornamentation covering full length, lacking germ slits or germ pores (Figs. 9-12).

**Etymology:** Referring to the host.

**Holotype:** HONG KONG, New Territories, Tai Po Kau Nature Reserve, on a dead culm of *Bambusa chungii*, 8 Sep. 1998, Dequn Zhou (HKU (M) 9143).

**Other material examined:** HONG KONG, New Territories, Tai Po Kau Nature Reserve, on a dead culm of *Bambusa chungii*, 8 Sep. 1998, Dequn Zhou (HKU (M) 9373).



**Figs. 1-12.** *Striatodecospora bambusae* (from holotype).
**1.** Appearance of ostiole of ascoma on host surface. **2.** Vertical section of ascoma. **3.** Ostiole. **4.** Paraphyses. **5.** Section of ascoma. **6.** Peridium. Note the inner part with hyaline, flattened cells and the outer part with dark brown, thick-walled, angular or compressed cells. **7-8.** Ascus with 8 ascospores. Note the J+, subapical ring in fig. 8. **9-12.** Ascospores with conspicuous, striate wall ornamentation (arrowed). Bar: 1 = 100 µm, 2 = 100 µm, 3 = 20 µm, 4 = 10 µm, 5 = 100 µm, 6 = 10 µm, 7 = 20 µm, 8 = 10 µm, 9-12 = 10 µm.

Table 1. Comparison of *Striatodecospora* with some related genera

| | *Striatodecospora* | *Spirodecospora* (Lu et al., 1998) | *Anthostomella* (Francis, 1975) | *Amphisphaerella* (Kirschsten, 1934; Hyde, 1995) |
|---|---|---|---|---|
| Ascomata | Deeply immersed in host, obpyriform with a central ostiole | Deeply immersed in host, obpyriform with an oblique or central ostiole | Immersed often under a clypeus, globose, subglobose, or conical, with a central ostiole | Superficial with only the base immersed, globose with a central ostiole |
| Asci | Cylindrical, short-pedicellate, with a J+, wedge-shaped, subapical ring | Cylindrical, pedicellate, with a J+, wedge-shaped, subapical ring | Broad cylindrical, pedicellate, with a J+/J-, discoid or wedge-shaped, subapical ring | Cylindrical, pedicellate, with a J+/J-, discoid or wedge-shaped, subapical ring |
| Ascospores | One-celled, brown, lacking a germ slit or germ pores, without a sheath | One-celled, olivaceous or brown, lacking a germ slit or germ pore, surrounded by a hyaline, fibrous mucilaginous sheath | Mostly one-celled, light brown, olivaceous or black, mostly with a germ slit, surrounded by a sheath or with mucilaginous appendages | One-celled, brown, with equatorial perm pores, often surrounded by a sheath |
| Wall of ascospores | Conspicuously covered by striate ornamentation | Conspicuously spirally arranged verrucose wall ornamentation | Mostly smooth-walled | Smooth-walled |

## DISCUSSION

*Striatodecospora* has many characters which are consistent with the Xylariaceae, and may be well placed here (Hawksworth et al., 1995). Similar genera include *Anthostomella*, *Amphisphaerella* and *Spirodecospora*. *Striatodecospora bambusae* cannot be confused with *Spirodecospora*, which has verrucose ascospores covered with spiral wall ornamentation, and larger ascospores (28-45 × 11-15 µm vs. 14-16 × 5-6 µm) surrounding by fibrillar sheath (Lu et al., 1998).

*Striatodecospora bambusae* differs from *Anthostomella* species as the ascospores are covered by conspicuous, striate ornamentation and lack germ slits, germ pores, sheaths, or mucilaginous appendages. *Anthostomella* is a species-rich and heterogeneous genus with more than 250 species (Francis, 1975; Lu et al., 1998). Hyde (1996) reported 42 species of *Anthostomella* from palms and *Pandanus*, including ten new species. In our understanding of *Anthostomella* species, ascospores lack any spiral or striate ornamentation, but often have a longitudinal germ slit and are surrounded by a gelatinous sheath.

*Striatodecospora* is distinct from *Amphisphaerella*, where the ascomata are initially immersed but become superficial with only the base immersed. Ascospores of *Amphisphaerella* are smooth, often surrounded by a sheath, and have equatorial germ pores (Kirschsten, 1934; Hyde 1995).

All genera mentioned above are compared in Table 1. A key to *Striatodecospora* and similar genera is provided.

### Key to *Striatodecospora* and similar genera

1. Ascomata superficial, with base only immersed, ascospores with equatorial germ pores-------------------------------*Amphisphaerella*
   Ascomata immersed--------------------------------------------------- 2
2. Ascospores with ornamentation-------------------------------------- 3
   Ascospores lacking ornamentation------------------------------------ 5
3. Ascomata forming in a stroma, asci clavate, ascospores having sparse irregular striations or verrucose------------------- *Cocoicola*
   Ascomata lacking a stroma, asci cylindrical------------------------ 4
4. Ascospores covered by striate ornamentation, lacking a mucilaginous sheath-------------------------------- *Striatodecospora*
   Ascospores covered by spiral ornamentation, with a mucilaginous sheath-------------------------------------------------- *Spirodecospora*

5. Ascomata forming in a stroma, ascospores having a central pallid band and polar appendage------------------------------ *Fasciatispora*
   Ascomata lacking a stroma, ascospores lacking a central pallid band------------------------------------------------------------------------ 6
6. Ascospores lunate, surrounded by an elaborate, layered sheath----- ------------------------------------------------------------------ *Nipicola*
   Ascospores cylindrical or ellipsoidal-------------------------------- 7
7. Ascospores hyaline or yellow, with a thin sheath and blunt appendages---------------------------------------------------- *Sabalicola*
   Ascospores brown------------------------------------------------------- 8
8. Ascospores thick-walled, reddish brown, with two polar germ pores------------------------------------------------------- *Pandanicola*
   Ascospores thin-walled, brown, often surrounded by a slimy sheath------------------------------------------------------- *Anthostomella*

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# NEW SPECIES OF BIPOLARIS FROM SOUTH AFRICA [1]

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**ABSTRACT** A new fungal species, *Bipolaris prieskaensis*, isolated from the debris of *Pistacia vera* L. in Prieska (Northern Cape Province of South Africa), is described and illustrated, its affinity to closely related species is discussed to show the distinguishing morphological features.

**KEY WORDS** *Bipolaris, Bipolaris prieskaensis,* new species

The genus *Bipolaris* was established by Shoemaker in 1959, conidia were described as fusoid, straight or curved, and germinating by one germ tube from each end. *Bipolaris maydis* (Nisik. & Miyake) Shoem. was selected as the type species. Fifty two species were included in the monograph "Graminicolous species of the hyphomycetes genera *Bipolaris*, *Curvularia*, *Drechslera*, *Exserohilum* and their teleomorphs " by Sivanesan (1987), *Bipolaris* and *Curvularia* were compared by using characters of conidial morphology, conidia germination, hilum structure, conidial septum and wall structure, conidial septum ontogeny in this book. Since then, nine new species ( Sisterna, 1989; Chiang, Leonard&Dyke, 1989; Peng & Lu, 1989; Alcorn, 1990; Sivanesan,1992) of *Bipolaris* and 4 new combinations (Muchovel & Carvalho, 1989; Alcorn,1991) have been described. In this paper, a new species is described from cultures

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which were isolated from the debris of *Pistacia vera* L. in Prieska, Northern Cape Province of South Africa. They were isolated on 2% malt-extract agar (MEA), transferred to tap water agar and wheat straw medium( Sivanesan, 1987) and exposed to near-ultraviolet light in an incubator (25°C, on a 12 H light/dark diurnal cycle). The morphological description of the species is based on 10 d old cultures on tap water agar (TWA)+wheat straw medium when conidia are mature. Measurements and microphotographs of conidia and conidiophores were taken from slide mounts in plain lactophenol.

***Bipolaris prieskaensis*** W. Q. Chen & W. J. Swart, sp. nov. Figs. 1-4

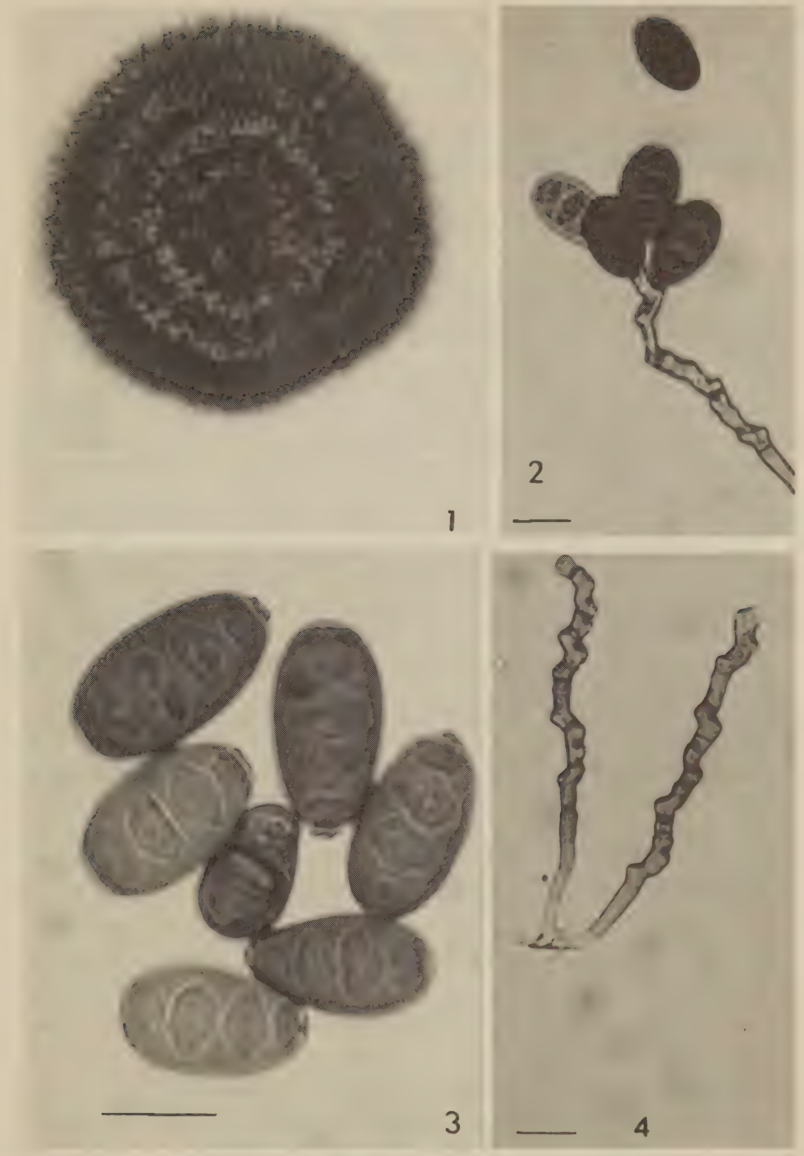
Etym.: Latin, *prieskaensis* = from Prieska, referring to the type locality.

Coloniae in agaro 'TWA+wheat straw" velutinae, floccosae, olivaceobrunneae vel brunneae. Hyhae septate, olivaceobrunneae vel brunneae, laeves. Conodiophora plerumque solitaria, recta vel flexuosa, saepe geniculata, simplicia vel ramosa, pallide brunnea vel brunnea, apicem versus pallidiora, usque ad 110 μm longa, 4-10μm crassa, 4-10 septate. Cellulae conidiogenae cylindrical, in conidiophores incorporatae, terminales vel intercalares, polyblasticae, sympodiales, cicatricae. Conidia acropleurogena, 3-4 distoseptata ( plerumque 3), recta, ellipsoidea, laevia, brunnea vel atrobrunnea, truncato hilo leviter protrudenti vel non protrudenti, 29-41×16-20μm.

Colony velvety, floccose, olivaceous brown to dark brown. Hyphae septate, olivaceous brown to brown, smooth. Conidiophores mostly solitary, straight in the lower sterile section, geniculate toward the apex, simple or branched, pale brown or brown, paler towards the apex, up to 110μm long, 4-10μm thick, 4-10 septate. Conidiogenous cells cylindrical, integrated, terminal and intercalary, polyblastic, sympodial, cicatrized. Conidia acropleurogenous, 3-4 distoseptate (mostly 3), straight, ellipsoid, smooth, mid to dark brown, with a hilum slightly protuberant up to 2.5μm diam or appearing non protuberant, 29-41×16-20μm.

Holotypus: PREM 56306 ( National Culture Collection of South Africa, Pretoria ), dried culture ex debris of *Pistacia vera* L., Prieska, Northern Cape Province, South Africa. T. D. Nieuwoudt, March 15, 1998, living culture ex-type, DN 123, deposited in the culture collection of the Department of Plant Pathology, University of the Orange Free State, Bloemfontein, South Africa.

As can be seen from the description and the illustration (Fig.1-4), the conidia vary little both in width and in length. Although superficially similar to *Bipolaris neergaardii* (Danquah) Alcorn, this species differs



Figs.1-4 Colony, conidia and conidiophores of *Bipolaris priskaensis* (Bars: Figs 2-4, 20µm). Fig. 1, colony; Fig. 2, conidiophores and conidia; Fig.3, conidia; Fig. 4, conidiophores.

from it in both the shape and size of the conidia. In *B. neergaardii* these tend to be broadly-ellipsoidal and much smaller (19-30×15-18μm) with inconspicuous hilum whilst in *B. preskaensis* they are most commonly ellipsoid and larger (29-49×16-20μm) with conspicuous hilum.

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# ACANTHOFUNGUS RIMOSUS GEN. ET SP. NOV., WITH REEVALUATION OF THE RELATED GENERA

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**ABSTRACT**: This article reports a new genus and new species *Acanthofungus rimosus*, which was collected at Taichung Prefecture in Taiwan. The generic and specific concepts of this new taxon are presented, based on studies of morphological and cultural characteristics. Separation among the genera *Acanthofungus*, *Acanthophysellum*, and *Xylobolus* is discussed. *Acanthophysium* is regarded as a synonym of *Xylobolus*. According to delimitations of the related genera, new combinations are proposed as follows: *Acanthofungus ahmadii*, *A. thoenii*, *Xylobolus apricans*, *Acanthophysellum bertii*, *A. canadense*, *A. cerussatum*, *A. dextrinoideocerussatum*, *A. lapponicum*, *A. measaverdense*, and *A. minor*.

**Key words:** *Aleurodiscus*, *Acanthophysellum*, *Acanthophysium*, *Gloeosoma*, Taiwan, *Xylobolus*.

In January of 1996 C.Y. Chien collected a wood-decaying basidiomycete from Taichung Prefecture in Taiwan. The fungus has a whitish and fairly large resupinate basidioma. It grew on the wood of a Taiwan endemic gymnosperm tree species, *Calocedrus formosana*, and had caused a white-pocket rot. The appearance of pulvinate and deeply cracked basidiomata is reminiscent of a *Xylobolus frustulatus* (Pers.:Fr.) Boidin. Chien prepared monosporous and polysporous cultures from this specimen, immediately after collecting it. Therefore, cultural studies including sexuality and nuclear behavior have been undertaken for studying this species. Later on, S.H. Wu received this specimen and its cultures from C.Y. Chien for studying its morphological and cultural features, in order to reveal its taxonomic placement. J. Boidin also has received a duplicate specimen from S.H. Wu, and has offered opinions regarding recognition of this new species, and discussion of its taxonomic placement. This specimen is herein treated by the authors as a new taxon *Acanthofungus rimosus* gen. et sp. nov., based on evidence derived from morphological and cultural characteristics. Recognition of this new genus has led us to reconsider the concept of some related genera which also possess acanthophyses and amyloid basidiospores. Some new combinations are proposed in this paper for species recognized in the genera *Acanthofungus*, *Acanthophysellum*, and *Xylobolus*.

## MATERIALS AND METHODS

*Morphological studies*. Free-hand thin sections of basidiomata were prepared for microscopic studies. For observations and measurements of microscopic characters, 5% KOH was used as a mounting medium to ensure rehydration. Sulphobenzaldehyde (SA) was used to detect the reaction of gloeocystidia, a bluish black color change indicating a positive reaction. Melzer's reagent (IKI) was employed to detect amyloidity and dextrinoidity. Cotton blue (CB) was used as a mounting medium to determine cyanophily.

*Isolation of monosporous and polysporous mycelia*. A small piece of fresh basidioma was attached to the underside of the lid of Petri dish using a small amount of glycerin as an adhesive. The hymenial surface of the piece was downward above a medium (1% malt extract agar) over night, so that the basidiospores could be discharged onto the agar surface. The lid was turned at an interval of 2 hrs to change several positions, in order to get several spore prints of comparatively low density on the agar surface. After 2 days, monosporous mycelia were obtained from the hyphae germinated from the spores by means of a fine needle under a dissecting microscope. Polysporous mycelium was derived by mixing mycelia derived from ca. 10 spores. The monosporous and polysporous mycelia were maintained in an agar medium (MEA), for undertaking various kinds of cultural studies.

*Cultural studies*. Cultural descriptions and species code diagnoses are from Nobles (1965) with amendments by Boidin and Lanquetin (1983). Nobles' code as detailed by Nakasone (1990) is adopted in this study. General methods of cultural studies have been previously described by Wu (1996). Terminology for nuclear behavior is based on Boidin and Lanquetin (1984).

## DESCRIPTION OF THE GENUS AND SPECIES

**Acanthofungus** Sheng H. Wu, Boidin & C.Y. Chien, *gen. nov.*

Basidiocarpus pulvinatus; superficies hymenialis plana. Systema hypharum monomiticum; hyphae fibulatae. Gloeocystidia SA+. Acanthophyses numerosae. Basidia clavata, 4 sterigmatibus. Basidiosporae ellipsoideae, laeves, tenuitunicatae, amyloideae. Ratio nucleata heterocytica.

Basidiomata pulvinate. Hymenial surface smooth. Hyphal system monomitic. Hyphae nodose-septate. Gloeocystidia SA+. Acanthophyses numerous. Basidia clavate, 4-strigmate. Basidiospores ellipsoid, smooth, thin-walled, amyloid, binucleate. Nuclear behavior heterocytic. Extracellular phenoloxidase –.

*Type species. Acanthofungus rimosus* Sheng H. Wu, Boidin & C.Y. Chien

*Etymology.* From *acantho* + *fungus*, referring to the presence of the acanthophyses of the fungus.

**Acanthofungus rimosus** Sheng H. Wu, Boidin & C.Y. Chien, *sp. nov.* (Figs. 1-7)

Basidiocarpus pulvinatus, mediocriter crassus; superficies hymenialis plana. Systema hypharum monomiticum; hyphae fibulatae. Gloeocystidia numerosa, tubularia vel cylindrica, 5-12 μm diam., SA+. Acanthophyses numerosae, 17-40 x 3-7 μm. Basidia clavata, 30-40 x 5.5-7 μm, 4 sterigmatibus. Basidiosporae ellipsoideae, laeves, tenuitunicatae, 6-8 x 4-5 μm, amyloideae. Ratio nucleata heterocytica.

Basidiomata pulvinate, at first forming small orbicular patches, then broadened and significantly thickened as a pulvinate form, up to 5 mm thick in section. The thickened basidiomata composed of numerous growth layers. The broadened basidiomata neighbor with adjacent ones; eventually the appearance of the basidiomata is deeply cracked, as if composed of many pieces. Basidioma attached to the substratum by a narrow base. Hymenial surface ivory-colored, smooth, farinaceous; margin abrupt. Abhymenial surface brown, glabrous, zonate. Context whitish. Hyphal system monomitic; hyphae nodose-septate. Subiculum composed of a thin basal layer, with compact texture; hyphae horizontal, glued together, colorless, 2.5-4.5 μm diam., thick-walled. Hymenium distinctly thickening, differentiated from the subiculum, with compact texture; hyphae mainly vertical, colorless, usually glued together and indistinct, narrower than those of subicular hyphae, thin- or slightly thick-walled. Crystal masses fairly abundant in the thickening subhymenium near hymenium. Gloeocystidia numerous, arranged in the thickening subhymenial palisade, tubular or cylindrical, may exceed 100 μm long, 5-12 μm diam., slightly thick-walled when young, with 0.6-1.2 μm thick walls when old, SA+, with many secondary septa in old gloeocystidia, colorless, but become brownish towards old hymenial surfaces and brown lateral sides of basidioma. Acanthophyses numerous, arranged in a palisade at hymenial surface and lateral sides, cylindrical or clavate, with obtuse or acute apices, bearing numerous 1-2 μm long aculei for apical halves, 17-40 x 3-7 μm (aculei excluded), slightly thick-walled towards apices, colorless, the brownish and thick-walled ones present in old hymenial surfaces and brown lateral sides. Basidia clavate, 30-40 x 5.5-7 μm, 4-sterigmate. Basidiospores ellipsoid, adaxially slightly concave, bearing a minute apiculus, smooth, thin-walled, with fairly homogeneous contents, colorless to brownish, 6-8 x 4-5 μm, IKI bluish black, CB −, binucleate.

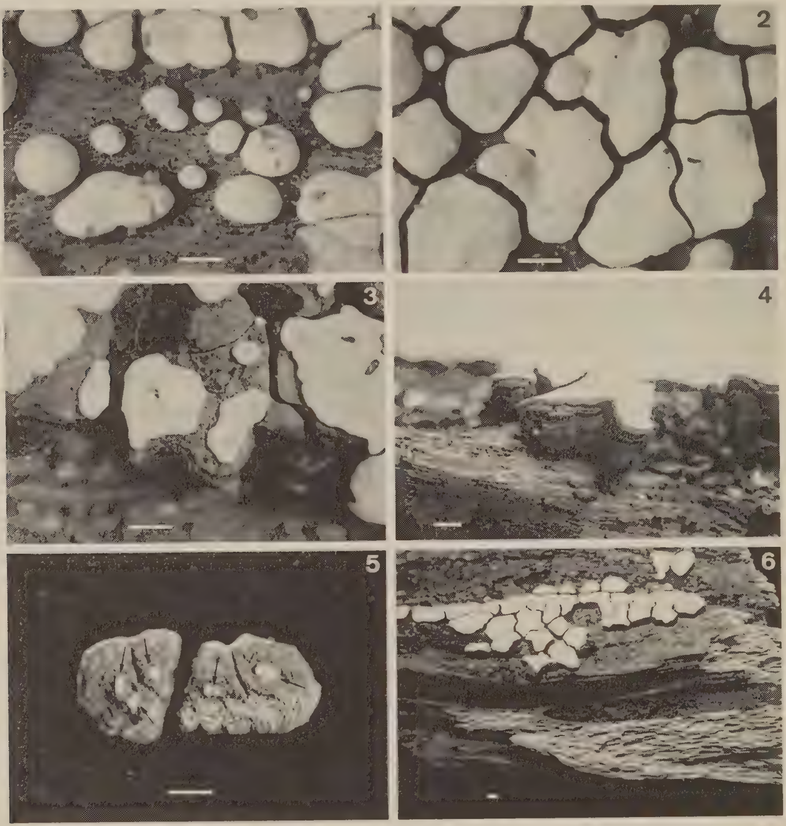
*Holotype.* TAIWAN. TAICHUNG PREFECTURE: Malin, alt. 660 m, on a white-pocket rot wood of *Calocedrus formosana*, leg. C.Y. Chien, 24-I-1996, *Wu 9601-1* (TNM).

*Etymology.* From *rimosus* (= full of cracks), referring to the general appearance of the hymenial surface.

*Specimen examined.* TAIWAN (see holotype).

*Distribution.* Known only from Taiwan.

*Cultural description* (polysporus mycelium from the holotype). 1 wk growth: Colony trace. Mat white. 2 wk growth: Colony radius 5-7 mm. Advancing zone bayed. Mat whitish. Aerial mycelium almost absent. Advancing hyphae colorless, nodose-septate, 1.7-3.5 $\mu$m diam., thin-walled. 3-5 wk growth: Colony radius respectively 8-12, 11-16, and 12-20 mm. 6 wk growth: Colony radius 13-26 mm. Advancing zone bayed. Mat slightly yellow. Aerial mycelium slightly pellicular or almost absent, plumose. Hyphal



Figs. 1-6. *Acanthofungus rimosus* (holotype). Fig. 1. Young basidiomata forming small orbicular patches. Fig. 2. Broadened basidiomata which neightbor with adjacent ones, look like a deeply cracked basidioma composed of many pieces. Fig. 3. Whitish new growth layer of the basidiomata arising from the brownish old layers. Fig. 4. Lateral view of the basidiomata, showing a whitish new growth layer on the surface and beneath numerous old brownish layers. Fig. 5. Abhymenial view of the basidiomata. The tiny bases represent small intitials of the basidiomata (arrows indicate the bases of the basidiomata). Fig. 6. The whitish basidiomata and the woody substratum, showing the white-pocket rot appeared in the wood. Scale bars = 2 mm.

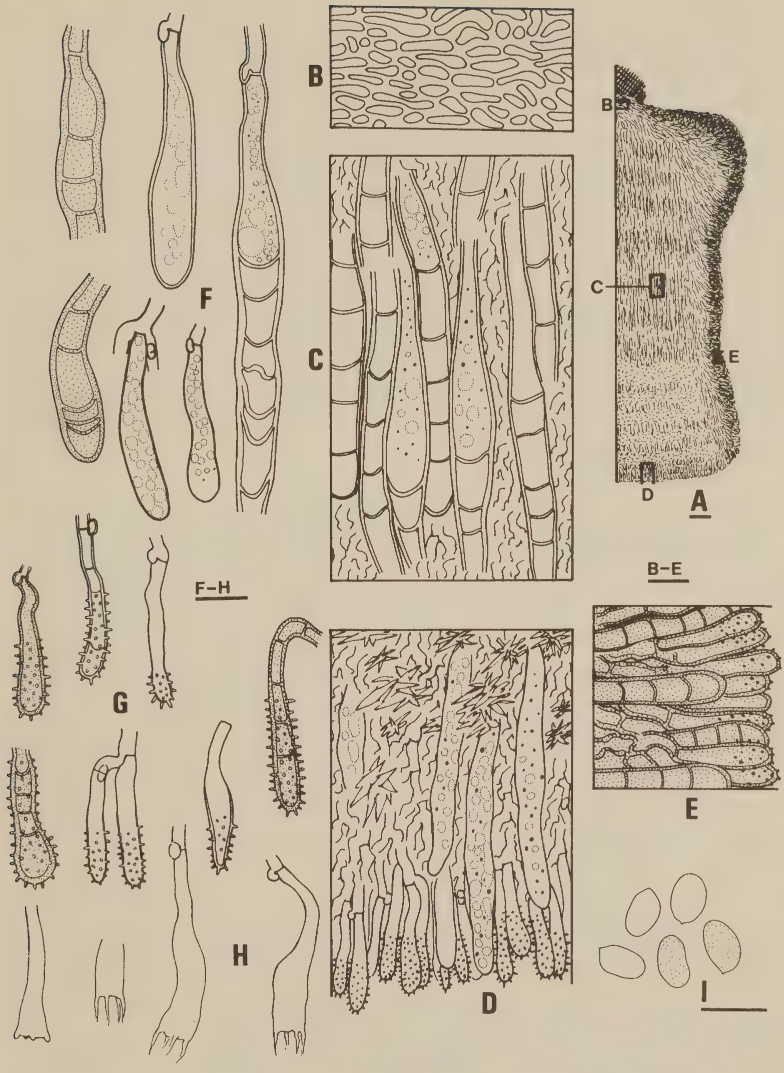


Fig. 7. *Acanthofungus rimosus* (holotype). A. Basidioma section. B. Section showing basal layer of the basidioma. C. Section showing a thickening hymenium, showing the gloeocystidia arranged in a palisade. D. Section of a hymenial portion. E. Section of a lateral edge of the basidioma, with the acanthophyses at the lateral surface. F. Gloeocystidia. G. Acanthophyses. H. Basidia. I. Basidiospores. Scale bar of A = 100 $\mu$m; scale bars of B-I = 10 $\mu$m.

system monomitic. Hyphae colorless, moderately ramified, nodose-septate, 1.7-3.5 μm diam., thin-walled. Rod-like crystals present. No distinct odor. Not fruiting.
*Oxidase reactions.* TAA: −, 0; −, 0. GAA: −, 0; −, 0. TYA: −, tr; −, 15-20.
*Species code.* 1. 3c. 7. 32. 36. 38. 47. 55. 58. 63.
*Sexuality.* Heterothallic. Three types of monosporous isolates have been classified based on the result of the test for determining its mating system (type I: 2, 4, 8, 10, type II: 3, 7, 11, type III: 1, 5, 6, 9, 12). Monosporous isolates of type I are compatible with type II, while those of type III are incompatible with either type I or type II. One of two possibilities may be present. First, this species may be tetrapolar, while the pole which compatible with type III is lacking among the tested monosporous isolates. On the other hand, this species may be bipolar in sexuality with type I and type II two opposite poles. If this is true, the type III could be regarded as a sterile element.
*Nuclear behavior.* Heterocytic. Basidiospores binucleate; monosporous mycelium usually uninucleate, rarely dikaryotic, terminal cells have more nuclei up to 5; polysporous mycelium dikaryotic.

## DISCUSSION

In possessing characters of pulvinate basidiomata, clamped hyphae, gloeocystidia, acanthophyses, and amyloid basidiospores, the new species proposed in this paper could be treated in the broad sense of the genus *Aleurodiscus* J. Schröt. However, delimitation of the genus *Aleurodiscus* is controversial among mycologists. Comparatively narrow concepts of generic names for accommodating members of *Aleurodiscus* s.l. have been presented, i.e. *Acanthobasidium* Oberw. *Acanthophysellum* Parmasto, *Acanthophysium* (Pilát) G. Cunn., *Aleurobotrys* Boidin et al., *Aleurodiscus* s.s., and *Gloeosoma* Bres. Many mycologists have adopted a narrow generic concept for *Aleurodiscus* (e.g., Boidin et al., 1985; Ginns and Lefebvre, 1993; Wu, 1995). However, some others (e.g., Hjortstam and Larsson, 1994; Núñez and Ryvarden, 1997) are inclined to use a broad sense of *Aleurodiscus*.

The genus *Acanthophysellum* could be considered for placement of our new species, owing to the presence of clamped hyphae, acanthophyses, and smooth basidiospores. However, the white-pocket rot and the phenoloxidase negative reaction of this species deviates from the white rot and phenoloxidase positive reaction in *A. lividocoeruleum* (P. Karst.) Parmasto, the type species of *Acanthophysellum* (Boidin et al., 1968). Moreover, nuclear behavior of *A. lividocoeruleum* is astatocoenocytic (Boidin et al., 1968). *Stereum ahmadii* Boidin shares many of the same features with the proposed new species, but is distinguished from the latter in having smaller basidiospores. *Acanthophysium thoenii* is also similar to the proposed new species and *S. ahmadii* by the same features, but differs in having larger basidiospores. Therefore, *Acanthofungus rimosus*, *A. thoenii*, and *S. ahmadii* should be placed in the same genus. In addition, *A. thoenii* and the proposed new species share some additional cultural features such as the slow growth of the mycelia, binucleate basidiospores, and heterocytic nuclear behavior. Cultural studies have not been undertaken for *S. ahmadii.*

The genus accommodating the three species shares similar characters of rot type and phenoloxidase reaction with the genus *Xylobolus* P. Karst., but in contrast have clamped hyphae and heterocytic nuclear behavior. Nuclear behavior of *Xylobolus* is holocoenocytic. The differences in the hyphal septation and nuclear behavior permit us to treat the three species in the new genus *Acanthofungus*. New combinations are proposed below.

***Acanthofungus ahmadii*** (Boidin) Sheng H. Wu, Boidin & C.Y. Chien, *comb. nov.*
Basionym: *Stereum ahmadii* Boidin, Biologia Lahore 2: 217. 1956.
≡ *Xylobolus ahmadii* (Boidin) Boidin, Rev. Mycol. 23: 341. 1958.

***Acanthofungus thoenii*** (Boidin, Lanq. & Gilles) Sheng H. Wu, Boidin & C.Y. Chien, *comb. nov*.
Basionym: *Acanthophysium thoenii* Boidin, Lanq. & Gilles, Bull. Soc. Mycol. France 101: 356. 1985.
≡ *Aleurodiscus thoenii* (Boidin, Lanq. & Gilles) Núñez & Ryvarden, Synop. Fung. 12: 139. 1997.

The type species of *Acanthophysium*, *Aleurodiscus apricans*, was originally stated by Bourdot (1910) to possess both smooth and asperulate basidiospores. Boidin (1985), Manjón et al. (1990), and Telleria and Melo (1995) respectively reported that basidiospores from France and Iberian Peninsula are smooth. Nevertheless, Núñez and Ryvarden (1997) reported that the basidiospores are asperulate based on studies of the type specimen. However, the lectotype of this species (*Galzin 4157*, PC) was designated by Boidin (1985), not the specimen (*Galzin 4153*) studied by Núñez and Ryvarden (1997). The features of simple-septate hyphae, smooth-walled basidiospores, holocoenocytic nuclear behavior, and phenoloxidase negative reaction (Boidin et al., 1968) of this species indicate that this species should belong to the genus *Xylobolus*. Therefore, *Acanthophysium* is treated here as a synonym of *Xylobolus*, and new combination for *A. apricans* is proposed below.

***Xylobolus apricans*** (Bourdot) Sheng H. Wu, Boidin & C.Y. Chien, *comb. nov.*
Basionym: *Aleurodiscus apricans* Bourdot, Rev. Sci. Bourbon. 27: 5. 1910.
≡ *Acanthophysium apricans* (Bourdot) G. Cunn., New Zealand Dept. Sci. Ind. Res. Bull. 145: 155. 1963.

Different concepts have been defined by mycologists for the genus *Acanthophysium* (Cunningham, 1963; Boidin et al. 1985; Núñez and Ryvarden, 1997), and many species have been placed in this genus. We may continue to consider the generic placement of other species apart from the type of *Acanthophysium*, after treating *Acanthophysium* as a synonym of *Xylobolus*. Most of these species may belong to the genus *Acanthophysellum*, or the genus *Acanthofungus*, depending on the differences of their rot type and phenoloxidase reaction. Accommodation of two species in *Acanthofungus* has been proposed earlier in this paper. Some new combinations for species placed in

*Acanthophysellum* are proposed below.

***Acanthophysellum bertii*** (Lloyd) Sheng H. Wu, Boidin & C.Y. Chien, *comb. nov.*
Basionym: *Aleurodiscus bertii* Lloyd, Mycol. Writ. 7: 1288. 1924.

***Acanthophysellum canadense*** (Skolko) Sheng H. Wu, Boidin & C.Y. Chien, *comb. nov.*
Basionym: *Aleurodiscus canadensis* Skolko, Can. J. Res. Ser. C, 22: 258. 1944.

***Acanthophysellum cerussatum*** (Bres.) Sheng H. Wu, Boidin & C.Y. Chien, *comb. nov.*
Basionym: *Corticium cerussatum* Bres., Fung. Trid. II: 37. 1892.
≡ *Acanthophysium cerussatum* (Bres.) Boidin in Boidin, Lanq. & Gilles, Bull. Soc. Mycol. France 101: 340. 1985.

***Acanthophysellum dextrinoideocerussatum*** (Mánjon, Blanco & Moreno in Moreno, Blanco & Mánjon) Sheng H. Wu, Boidin & C.Y. Chien, *comb. nov.*
Basionym: *Aleurodiscus dextrinoideocerussatus* Mánjon, Blanco & Moreno in Moreno, Blanco & Mánjon, Mycotaxon 39: 351. 1990.

***Acanthophysellum lapponicum*** (Litsch.) Sheng H. Wu, Boidin & C.Y. Chien, *comb. nov.*
Basionym: *Aleurodiscus lapponicus* Litsch., Ann. Mycol. 42: 11. 1944.
≡ *Acanthophysium lapponicum* (Bres.) Boidin in Boidin, Lanq. & Gilles, Bull. Soc. Mycol. France 101: 340. 1985.

***Acanthophysellum measaverdense*** (Lindsey) Sheng H. Wu, Boidin & C.Y. Chien, *comb. nov.*
Basionym: *Aleurodiscus measaverdensis* Lindsey, Mycotaxon 30: 433. 1987.

***Acanthophysellum minor*** (Pilát) Sheng H. Wu, Boidin & C.Y. Chien, *comb. nov.*
Basionym: *Aleurodiscus cerussatus* (Bres.) Höhn. & Litsch. var. *minor* Pilát, Ann. Mycol. 24: 215. 1926.

So far we can not judge for many species whether they should belong to *Acanthofungus* or *Acanthophysellum*, due to the lack of knowledge regarding their phenoloxidase reactions. Cultural studies are needed for these species.

## ACKNOWLEDGMENTS

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# HETEROMYCOPHAGA TREMELLICOLA FOUND IN THE NEOTYPE SPECIMEN OF TREMELLA BRASILIENSIS

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**Abstract.** The mycoparasitic species, *Heteromycophaga tremellicola*, was found growing in the neotype specimen of *Tremella brasiliensis*. Conidiophores with haustoria and conidia in chains were discovered and investigated.

**Key words:** *Heteromycophaga glandulosae*, *Heteromycophaga tremellicola*, *Tremella brasiliensis*, conidiophores, haustoria

***Heteromycophaga tremellicola*** P. Roberts, Kew Bulletin 53(3): 631 (1998)

Conidiomata mixing in the basidiocarps of *Tremella brasiliensis*. Conidiophores (Fig. 2) hyphae shape, tubular, 2-4 µm in diameter, gelatinous thick-walled like, with clamp connections, each branch producing one or more chained conidia (Fig. 2). Conidia various in length, ellipsoid, fusiform, oblong to cylindrical, (9-)12-20(-22) x 4-6(-8) µm [$Q^{**}$=(1.38-)1.71-3.4(-5.5)], when detached, the clamp usually remains on one end while the other end leaves a trace (Fig. 3). Clamydospores-like are variable in size, (13-)15-24 x (10-)14-18 µm, globose to oval, thin-walled when young (Fig. 4), thick-walled when mature (Fig. 5)

Growing in the basidiocarps of neotype specimen of *Tremella brasiliensis*, BPI 718395 (selected by Bandoni, 1957),

Habitat and substrate: on the cortex of dead branches.

Locality: Brazil, Blumenau.

Distribution: Brazil, Panama.

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[*] Part of 181 of a series "Studies in Heterobasidiomycetes".

[**] Q value = length / breadth ratio (Bas, 1969)

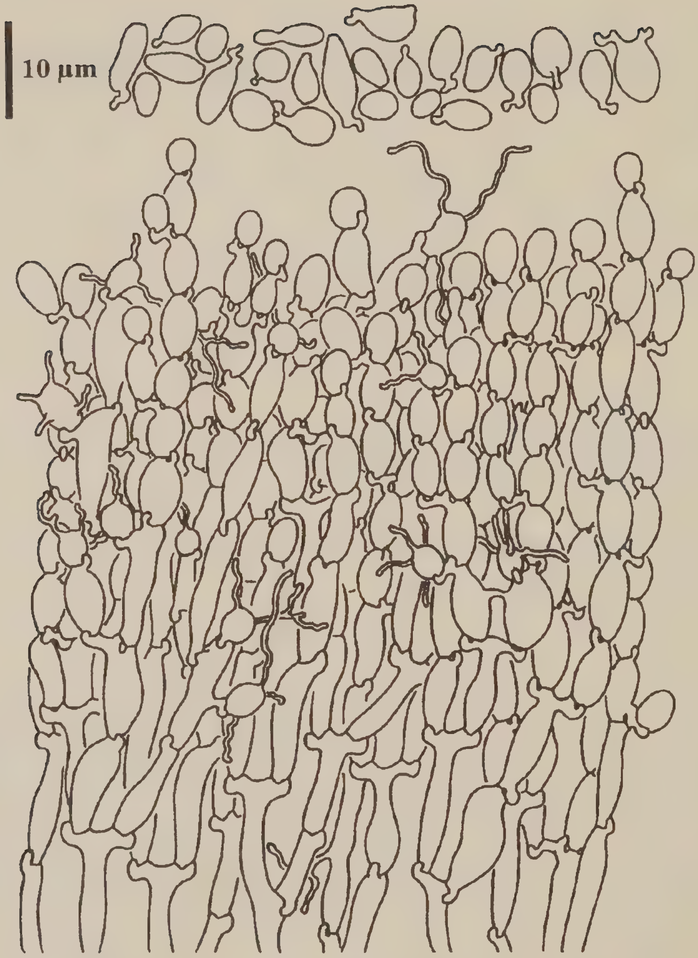

Fig. 1. Conidiophores of *Tremella brasiliensis* BPI 718395 (neotype) producing clamped conidia in chains, branched haustorial filaments and detached conidia of various shapes.

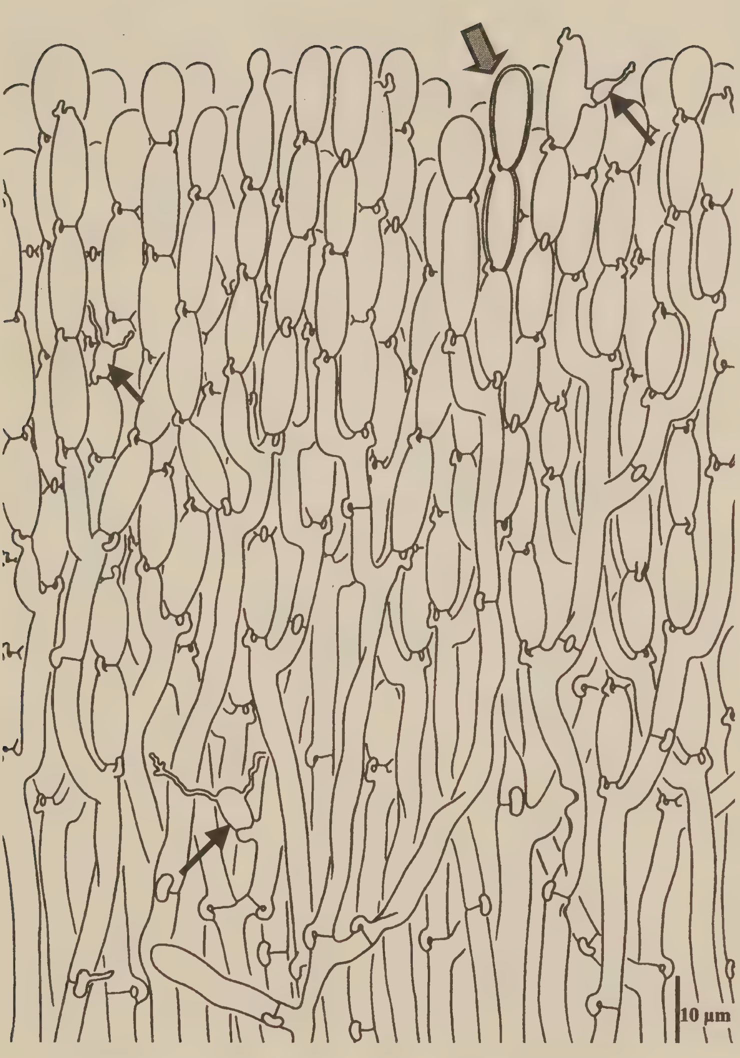

Fig. 2. Conidiophores of *Heteromycophaga tremellicola* producing conidia in chains. Bold arrows indicate haustoria arising from hypha and conidia. The conidia are occasionally slightly thick-walled (hollow arrow).

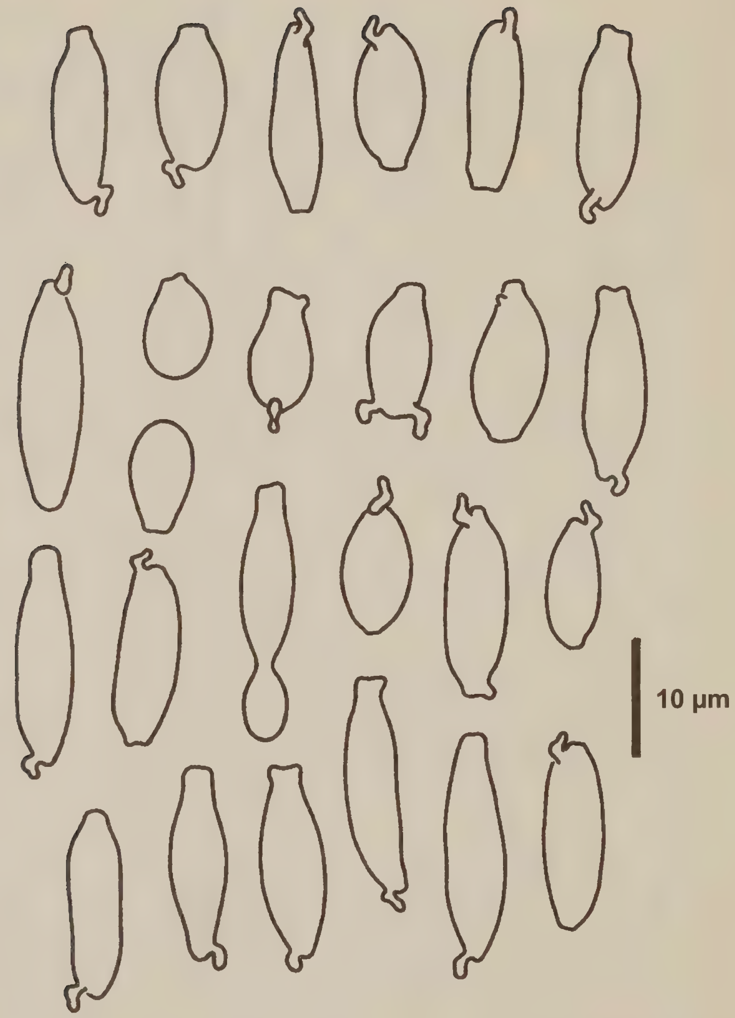

Fig. 3. Variation in conidial morphology of *Heteromycophaga tremellicola*. The appendage and clamp(s) indicate their growth in chains.

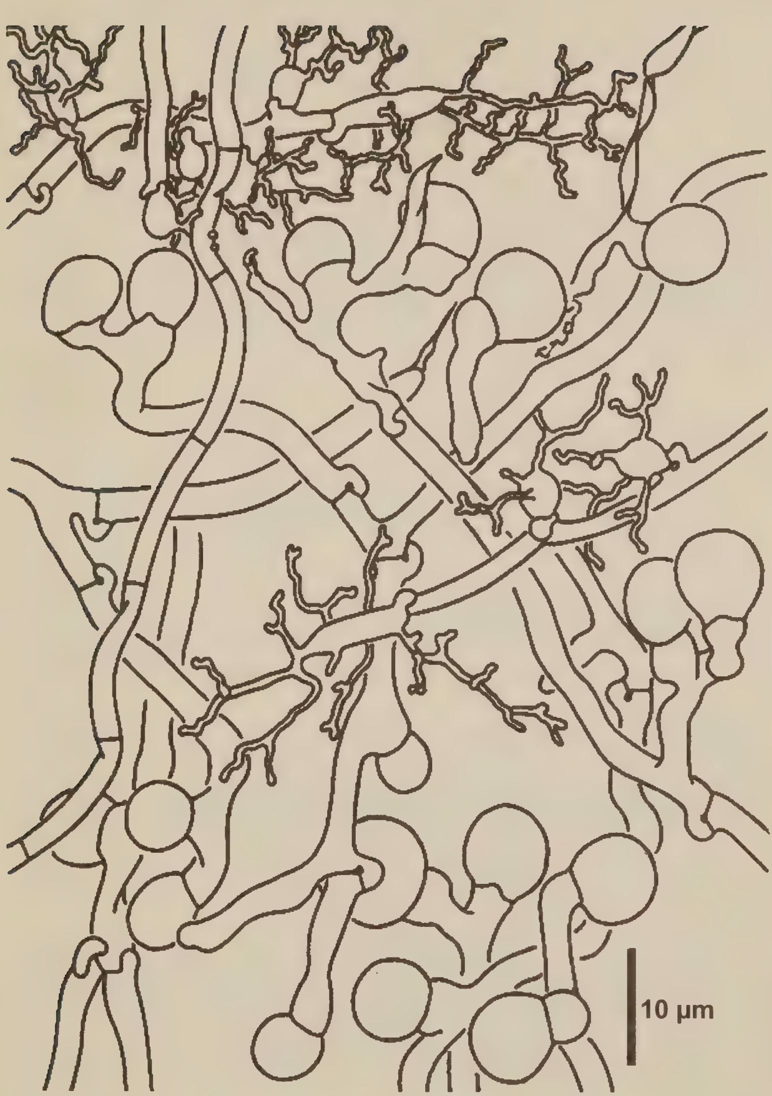

Fig. 4. Young terminally swollen cells of *H. tremellicola* growing in the lower part of *Tremella brasiliensis* basidiocarps close to the substratum. Note that haustoria with branched filamentous hyphae belong to *Tremella* cells.

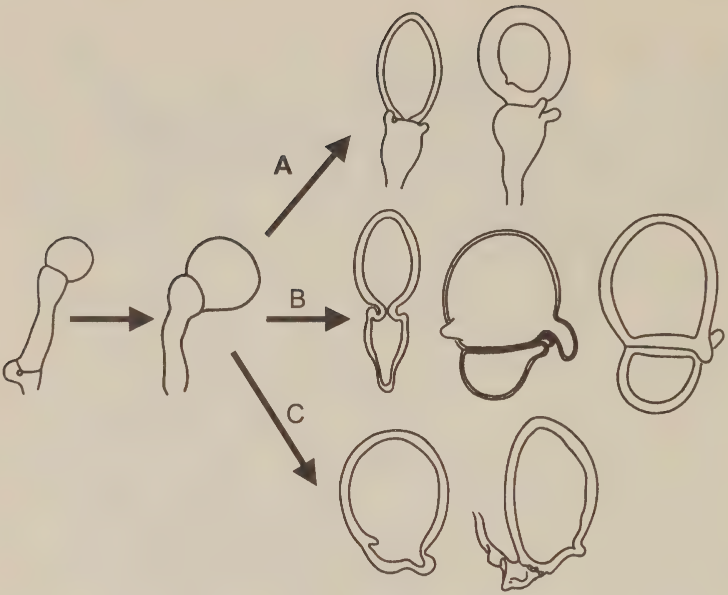

Fig. 5. Ontogeny of swollen cells in *Heteromycophaga tremellicola*. A, forming a single cell terminally. B, forming chlamydospore-like double cells. C, forming a single cell through fusion of two cells. Note the markings indicating two fusion cells.

Roberts (1997) published a new taxon, *Heteromycophaga glandulosae* P. Roberts, growing as galls on the basidiocarps of *Exidia glandulosa* Bull. He found two important morphological characters, conidiophores and irregularly swollen distorted hyphae, appearing in the conidiomata. A second taxon, *H. tremellicola*, was found growing in collections of *Tremella brasiliensis* from Brazil and Brunei (Roberts & de Meijer, 1997; Roberts & Spooner, 1998). In both species of *Heteromycophaga* conidia were said to be produced terminally, singly, or in chains, but haustorial cells were not seen, although the two taxa were assumed to be parasites.

The type specimen of *Tremella brasiliensis* was restudied by Bandoni (1957), but its anamorphic conidial stage was not seen. In our study of the type specimen, conidial structures were detected. By careful preparation of slides for microscopy, two kinds of conidiophores were clearly distinguished by the size and shape of their conidia (Figs. 1 - 2). One is the conidial stage of *H. tremellicola* (Fig. 2) while the other belongs to *T. brasiliensis* (Fig. 1). Comparison with illustrations of *Heteromycophaga* (Roberts, 1997; Roberts & Spooner, 1998) shows that Fig. 2 is identical with the conidial stage described for *H. tremellicola*. However, the detached conidia with one or more clamp(s) at one end and an indentation or appendage at the other (Fig. 3) indicate that they grow in chains. The haustoria arising from the hyphae of conidiophores and conidia were observed for the first time.

Some irregularly swollen cells were detected in the lower parts of basidiocarps (Fig. 4), but their function is not clear. In *Heteromycophaga glandulosae* they were assumed to be the points of interaction between parasite and host (Roberts,1997), but there is no clear evidence to support this. Their ontogeny (Fig. 5) indicates that the swelling of the terminal cells may represent thick-walled chlamydospore-like bodies.

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# 從新模式標本 Tremella brasiliensis 中發現 Heteromycophaga tremellicola

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重新研究 Lloyd 的新模式(neotype)標本 *Tremella brasiliensis* 發現混著另一種真菌 *Heteromycophaga tremellicola*，此種真菌的分生孢子柄具有吸器以及產生鏈狀的分生孢子為首次發現。

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# TAXONOMIC IMPLICATIONS OF UNUSUAL ZOOSPORE DISCHARGE IN AN ISOLATE OF SAPROLEGNIA UNISPORA

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## ABSTRACT

We have isolated a strain of *Saprolegnia unispora* whose primary zoospores exhibit a range of motilities extending from slow quivering to moderately rapid swimming depending on their sporangium of origin. Secondary spores swim normally suggesting that different subcellular mechanisms may control primary spore swimming than control that of secondary spores. A digitized video tape of this spore behavior can be viewed on the first author's web page (http://www.uncwil.edu/people/padgett). This behavior is similar to that previously reported for at least two species of *Isoachlya* suggesting that this genus, formerly brought into synonymy with *Saprolegnia*, might warrant reinstatement after further study.

**Keywords:** *Saprolegnia, Isoachlya,* zoospore discharge, water mold, motility.

We recently have isolated a unique Oomycete that brings into question the generic distinction between *Saprolegnia* and *Isoachlya*. Kauffman (1921) erected the saprolegniaceous genus *Isoachlya* to include the type species *I. toruloides* Kauffman and Coker and two new combinations: *I. paradoxa* (Coker), and *I. monilifera* (de Bary). Although his formal description of zoospore discharge in the type species indicated behavior as in *Saprolegnia* Nees, he considered the cymose mode of zoosporangial renewal, which is more typical of *Achlya,* to be sufficient justification for erecting the new genus.

Despite characterizing discharge for *I. toruloides* as being like *Saprolegnia,* Kauffman's (1921) narrative of planont behavior revealed that primary zoospores swam sluggishly by "curious somersaulting". He further noted that they encysted after about 20 minutes but that cysts never released actively swimming secondary spores. Coker (1923) later recognized *Isoachlya* in his monograph of the Saprolegniaceae and indicated (although not in the formal diagnosis) that when cultured at 79° F primary spores of his North Carolina isolate of *I. toruloides* were "expelled with force in a semitorpid state, gather(ed) strength after a few seconds of slow movement in an open cluster and sw(am) off". He interpreted the initial lethargic swimming as evidence that spore discharge resulted from hydrostatic pressure rather than rapid motility. Coker's (1923) isolate differed further from Kauffman's in that spore cysts liberated active secondary planonts.

Coker's (1923) monograph included the description of a new species, *I. eccentrica* Coker, also having sluggish primary zoospores. He characterized them as "emerging rather slowly with the cilia directed backward, then reversing and swimming sluggishly for a very short time, many coming to rest in the immediate neighborhood of the sporangium".

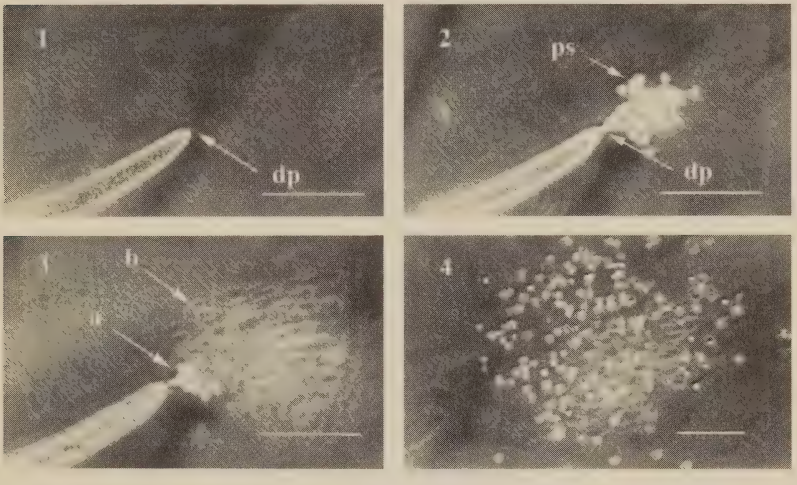
Seymour (1970) later expanded the genus *Saprolegnia* to include all described species of *Isoachlya* based on his observation that cymose sporangial renewal was not uncommon within the former. Since this character had been the hallmark of Kauffman's generic circumscription, Seymour's action appears justified. Seymour, however, made no mention of having seen living cultures of *Isoachlya* exhibiting primary zoospore sluggishness or of considering this characteristic in his revision.

We have isolated a water mold from local, non-saline soil that conforms very closely to Seymour's (1970) account of sexual characteristics in his new combination of *Saprolegnia unispora* (= *I. unispora)* Coker & Couch. It's primary zoospores, however, exhibit sluggish motility reminiscent of that described for: *I. toruloides* (Kauffman ,1921; Coker 1923), *I. unispora* (Coker, 1923), and *I. eccentrica* (Coker, 1923). We have maintained this single spore isolate in axenic water culture both on sesame and hemp seeds for more than three years and have found the primary zoospore behavior described below to be consistent through repeated subcultures.

We have observed that all primary spores from a single sporangium exhibit similar behavior, but are puzzled by the fact that spores from different sporangia even within the same subculture can exhibit a range of motilities. The extremes of this range extend from slow quivering followed by rapid encystment (Figs. 1 - 4) to momentary sluggishness followed by moderately rapid swimming. The former is analogous to Kauffman's (1921) account of *I. toruloides* (except for the duration of sluggish movement prior to encystment) while the latter is like that described by Coker (1923) for the same species. This variability within a single isolate seems particularly curious because our culture was derived from a single spore. We wonder, however, if the differences between Kauffman's (1921) and Coker's (1923) accounts of primary spore behavior in *I. toruloides* (given above) could have resulted from their observing sporangia representing the extremes of this discharge range within cultures similar to our own.

Despite the lack of a genetic explanation for this curious behavior, we feel that our isolate could represent a valuable experimental tool for scientists interested in investigating the subcellular control of motility. Accordingly, we will deposit it at the American Type Culture Collection to facilitate its availability.

Typical zoospore behavior for this isolate can be seen only in water culture in a Petri dish. We have found that standard wet mounts on microscope slides force premature encystment of primary spores and otherwise distort the "spore cloud" effect described below. This fact has made it difficult to obtain sharply focused stop-action micrographs of the discharge process. Figures 1 through 4 depict sequential stages of discharge of a single sporangium whose primary spores represent the lower end [i.e. least motility before encystment (LMBE)] of the motility spectrum for this isolate. The microscope is focused on the discharge pore in Figs. 1 through 3. Note particularly that in Fig. 2 (early discharge) all spores are roughly in focus whereas in Fig. 3 only those most recently discharged (region "a" adjacent to the discharge pore) are in focus while those farther away (region "b", earliest discharged) have settled downward out of the focal plane. Minimal spore motility precluded their movement away from the vicinity of the exit pore except by gravity or passively in water currents. Consequently, the spores slowly settled as a group, which we refer to as a "spore cloud", through the water column until they came to rest as a discrete cluster on the bottom of the culture dish (Fig. 4). We believe that this "spore cloud" is analogous to what Coker (1923) described as an "open cluster" for *I. toruloides.* It is simple to estimate the relative number of sporangia in a given subculture that ejected LMBE spores by counting the discrete primary spore cyst clusters scattered over the bottom of the dish. Care must be taken however, to avoid agitating the dish because cyst clusters can be dispersed by fluid currents.



Figs. 1 – 4. Sequential, still-frame, phase contrast micrographs of a single LMBE-type sporangium discharging primary spores. Scale bar for all figures is 100 µm. 1. Undischarged sporangium, arrow indicates discharge papilla (dp). 2. Early stage of discharge, arrows indicate discharge papilla (dp) and primary spores (ps). 3. Mid-discharge, note that most recently discharged spores (a) are still in the focal plane of the discharge papilla while those released earlier (b) have settled below the focal plane. 4. Encysted spore cloud resting on bottom of culture dish.

We also have documented discharge of an LMBE-type sporangium on videotape and have posted a digitized version on the first author's web page (http://www.uncwil.edu/people/padgett). Viewers with access to high speed Internet connections should view the "Real Video" (higher resolution) version of this video because the last frame shows the slowly rocking ("quivering") primary spores just before encystment. We have observed that these are typically shaped (pyriform) biflagellate zoospores whose flagella beat too slowly to permit normal motility.

Primary spore cyst morphology appears typical of other saprolegniaceous protists, and the average encystment duration is about three hours. Secondary laterally biflagellate, reniform planonts emerge from a high percentage of cysts at the end of this resting period and all exhibit typical rapid motility after a brief period (1 to 15 minutes) of lethargic rocking near the cyst exit pore. We are intrigued by the possibility that differences in motility between primary and secondary spores in our isolate could suggest that there are different subcellular regulators for motility in primary spores than in secondary ones.

We are of the opinion that the taxonomic significance of lethargic primary spore motility should be reevaluated within the genus *Saprolegnia.* This would be particularly timely in light of a recent phenetic analysis of the Saprolegniaceae (Powell and Blackwell, 1997) which showed *Isoachlya* to be clearly separated from *Saprolegnia.*

Daugherty *et al.* (1998) recently reported results of a phylogenetic comparison of selected saprolegniaceous genera based on ITS gene sequencing and indicated that *Saprolegnia* is ancestral both to *Achlya* and *Thraustotheca.* They expressed agreement with Salvin (1941) that this ancestry suggests evolutionary suppression of primary-type zoospores. Morphological evidence presented in the present paper suggests that lethargic primary zoospore swimming and rapid encystment might position our isolate between *Saprolegnia* and *Achlya* in this evolutionary continuum (Salvin, 1941). It would be valuable to determine if ITS sequence data both for our isolate and other species of the former genus *Isoachlya* support this intermediate positioning and justify reseparation of *Isoachlya* from *Saprolegnia.*

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# POLYPOROID WOOD-ROTTING FUNGI (BASIDIOMYCETES) II - NEW RECORDS FROM SOUTHERN BRAZIL

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## ABSTRACT

A survey of polyporoid fungi in wood from Santa Catarina State ($26^0$ 00' to $30^0$ 00' S lat, $48^0$ 30' to $54^0$ 00' W long) and Paraná State ($22^0$ 30' to $26^0$ 45' S lat, $48^0$ 00' to $54^0$ 30' W long) resulted in new records of Basidiomycetes (Phragmo- and Holobasidiomycetidae). Of them 14 are new records for Santa Catarina State, and 5 for Paraná State. *Aporpium dimidiatum*, *Phellinus grenadensis*, *Megasporoporia setulosa* and *Skeletocutis diluta* are new records for Brazil, and *Rigidoporus amazonicus* is recorded by the second time in the world. Two species (*Henningsia brasiliensis* and *Hydnopolyporus fimbriatus*) are new records for Santa Catarina Island.

**KEY WORDS**: polyporoid fungi, biodiversity, Polyporaceae, taxonomy, Basidiomycetes.

## INTRODUCTION

The mycota of Santa Catarina State has been studied in the last years, extending our knowledge about this interesting group of organisms. Periodical collections have been undertaken in this area and in the neighbour Paraná State, Some surveys resulting in new records, and on some occasions, in new species (Loguercio-Leite & Wright, 1991; 1998; Loguercio-Leite et al, 1998). Mainly the collections have been realized in forests in a secondary stage (Atlantic Rain Forests) through these States. The scope of this survey is to contribute to a better knowledge of polypores on subtropical regions.

## MATERIALS AND METHODS

Collections were undertaken during recent years on different regions of Santa Catarina (SC) and Paraná (PR) States. The collections considered in this study are deposited on Herbarium FLOR, and were compared with materials of FLOR (Florianópolis, Brazil), as well as BAFC (Buenos Aires, Argentina). Herbarium designations are those Holmgren et al. (1990). Measurements and drawings were made from freehand sections mounted in 1% phloxine solution + 5% KOH solution and Melzer's reagent. Colours are those from Munsell (1975).

Distribution data were obtained from different authors, referring to South America, during the 20th century.

## RESULTS AND DISCUSSION

The material was identified and included in different orders, we present them according the systematic arrangement in Hawksworth et al., 1995. Illustrations of spores are presented (Fig. 1).

### Aporpiaceae

*Aporpium dimidiatum* A. David
Description in David, 1974.
Remarks: basidiomata resupinate, efuse-reflexd to dimidiate, on palms, with a strong anise odour on the field, hymenial surface white when fresh, pinkish when touched, these characters have not been cited previously, our materials present (3-) 5-7 (-9) pores/mm, spores oblong-elliposid (4-) 4.5-6 x 3-3.5 (-4) µm very similar with data from David (1974) and Setliff & Ryvarden (1982). Reid (1992) tranfered this species to *Elmerina,* in a new combination, *E. dimidiata* (David) Reid. New record for Brazil .
Distribution: not found before in South America.
Material examined: BRAZIL, SC, Ilha de Santa Catarina, Canto dos Araças, Gerber, A. Neves, M. A. & Althoff, S. 795, 25/X/95 (FLOR 11356); ibid, ipse 813, 22/XI/95 (FLOR 11357); ibid, ipse 816, 19/XII/95 (FLOR 11358); GUADALOUPE, Forrestiere de Grosse, David, A. LY-AD 2158, 30/X/1976 (FLOR 11418).

*Aporpium substuppeus* (Berk. & M. A. Curtis) Rajchenb.
≡ *Polyporus substuppeus* Berk. & M. A. Curtis. J. Linn. Soc. 15: 380, 1876.
Description in Rajchenberg, 1987.
Remarks: pores irregular, angular to daedalioid, 1-2 per mm, spores ovoid 5-6.5 (-7.5) x 3-4.5 µm. Rick described it as *Polyporus gregarius* for Rio Grande do Sul State (Rajchenberg, 1987). Ryvarden (1991) proposed the combination *Protomerulius substuppeus* (Berk. & Curt) Ryv. New record for Santa Catarina State.
Distribution: Brazil [Rio Grande do Sul (Rajchenberg, 1987), Amazonas (holotype - Trail)].
Material examined: BRAZIL, SC, Ilha de Santa Catarina, Morro da Lagoa da Conceição, Gerber, A., Loguercio-Leite, C. & Cabral, R. 377, 23/II/1994 (FLOR 11398).

### Ganodermataceae

*Humphreya coffeatum* (Berk.) Steyaert
≡ *Polyporus coffeatum* Berk. Ann. Mag. nat. Hist., 3: 385, 1839.
Description in Steyaert, 1972.
Remarks: spores of our materials are longer 12-14(-15) x 7-9 µm than Steyaert's data (9.5 – 11.5 x 6.5 -8 µm). New record for Santa Catarina and Paraná States.
Distribution: Brazil [Bahia (Góes-Neto, 1999)]; Argentina (not published)
Material examined: BRAZIL, SC, Morro Lagoa da Conceição, Halmenschlager, M. A., Neves, M. A. & Degenhardt s/n, 22/IX/1994 (FLOR 11148); PR, Capanema, Basso, J. 990, 04/III/1997 (FLOR 11493); ARGENTINA, Misiones, P.N. Cataratas del Iguazú, Wright, Rajchenberg & Job, M3625, 7/IV/1985 (BAFC 30031).

## Hymenochataceae

*Aurificaria luteo-umbrina* (Romell) D. A Reid
≡ *Phaeoporus luteo-umbrina* Romell, Svenska Vetensk. Akad. Handl. 26 (16): 27, 1901.
Description in Reid, 1963.
Remarks: 5-7 pores/mm, tramal setae ventricose, straight apex, 35-80 X (5-) 7-9 µm; spores ovoid to subglobose, hyaline thin to slightly thick-walled, 4-4.5 X 3-3.5 µm similar to those of Reid (1963). New record to Santa Catarina State.
Distribution: Brazil [Mato Grosso (Ryvarden, 1974)]; Argentina (Reid, 1963).
Material examined: BRAZIL, SC, Ilha de Santa Catarina, Morro Lagoa Conceição, Neves, M. A., Halmenschlager, M. A. & Degenhardt, R. 540, 22/IX/1994 (FLOR 11380); ibid, Gerber, Foresti & Althoff, 666, 30/V/1995 (FLOR 11387), ibid, ipse, Gerber, Neves & Althoff 780, 23/VII/1995 (FLOR 11388).

*Phellinus bambusarum* (Rick) M. J.. Larsen
≡ *Poria bambusarum* Rick, Broteria ser. Cienc. Nat. 6:146, 1927.
Description in Larsen & Cobb-Poulle, 1990.
Remarks: material examined present 7-9 pores/mm, setae straight, ventricose 20-26 x 7-7.5 µm, spores globose to subglobose, hyaline, slightly thick-walled, brown in IKI, 4.2-5 (-5.5) X 4-5 µm. New record for Santa Catarina State.
Distribution: Brazil [Paraná (Rajchenberg & Meyer, 1990); Rio Grande do Sul (Rajchenberg, 1987)].
Material examined: BRAZIL, SC, Ilha de Santa Catarina, Morro da Lagoa da Conceição, Gerber, A.L., Althoff, S. & Neves, M.A. 735, 26/VII/1995 (FLOR 11263)

*Phellinus grenadensis* (Murrill) Ryvarden
≡ *Pyropolyporus grenadensis* Murrill, North Amer. Flora 9:107, 1908.
Description in Larsen & Cobb-Poulle, 1990.
Remarks: our material presents 4-5 pores per mm; ovoid to ellipsoid spores, with brown walls, (4.5-) 5 (-5.8) x 3.5-4 µm similar to those of Larsen & Cobb-Poulle (1990). New record for Brazil.
Distribution: Venezuela (Fidalgo, 1968b).
Material examined: BRAZIL, SC, Ilha de Santa Catarina, Morro Lagoa da Conceição, Willerding, A. & Santos, B. 420, 02/IV/1994 (FLOR 10920); Santo Amaro da Imperatriz, Atanazio, J. & Willerding, A. 450, 20/V/1994 (FLOR 10928).

*Phellinus fastuosus* (Lév.) Ryvarden
≡ *Polyporus fastuosus* Lév., Ann. Sci. Nat. Bot. 2:190, 1844.
Description in Larsen & Cobb-Poulle, 1990.
Remarks: 5-8 pores/mm; generative hyphae 2-2.5 µm, skeletal hyphae 3.5-5 µm (trama), 3.5-4 µm (context); spores subglobose thick-walled, gold brown, 4.5–5 (-5.5) X 4-4.5 (-4.8) µm. New record for Paraná State.
Distribution: Brazil [Bahia (Góes-Neto, 1999)]; Venezuela (Rodriguez et al. 1995); Colombia (Setliff & Ryvarden, 1983); Paraguay (Popov & Wright, 1998).
Material examined: BRAZIL, PR, Fazenda São Luiz, Capanema, Basso, J. 994, 995, 04/III/1997 (FLOR 11487, 11488).

## Coriolaceae

*Coriolopsis polyzona* (Pers.) Ryvarden
≡ *Polyporus polyzonus* Pers. Gaudichaud Voy. aut. Monde, Bot., 170, 1827.

Description in Ryvarden & Johansen, 1980.
Remarks: the material presents 2-3 pore..s/mm; spores cylindric, hyaline, thin-walled, smooth, 7-8 x 2 µm similar to Ryvarden & Johansen's data (1980). New record for Santa Catarina State.
Distribution: Brazil [Bahia (Góes-Neto, 1999); Paraná (Rajchenberg & Meyer, 1990)]; Argentina and Venezuela (Fidalgo, 1968b); Paraguay (Popov & Wright, 1998).
Material examined: BRAZIL, PR, Capanema, Basso, J. 997, 04/III/1997 (FLOR 11495); SC, Blumenau, Althoff, S. s/n, 07/IV/1997 (FLOR 11717).

*Datronia caperata* (Berk.) Ryvarden
≡ *Polyporus caperatus* Berk. Ann. Mag. Nat: Hist. Ser. 1, 3:391, 1839.
Description in Ryvarden & Johansen, 1980.
Remarks: 3-4 pores/mm; spores cylindric, hyaline, thin-walled, smooth, 8-10 x 3-3.5 (-4) µm wider than Ryvarden & Johansen's data (1980). New record for Paraná and Santa Catarina States.
Distribution: Brazil [Bahia (Góes-Neto, 1999); São Paulo (Gugliota & Capelari, 1995, Gugliota, 1997)]; Venezuela (Fidalgo, 1968b, Rodriguez et al. 1995); Paraguay (Popov & Wright, 1998).
Material examined: BRAZIL, PR, Capanema, Basso, J. 985, 04/III/1997 (FLOR 11486); ibid, SC, Ilha de Santa Catarina, Ponta das Almas, Villar, L., 1132, 30/XI/1997 (FLOR 11 665).

*Earliella scabrosa* (Pers.) Gilb. & Ryvarden
≡ *Polyporus scabrosus* Pers. in Gaudich. Voy. aut Monde p. 172, 1827.
Description in Wright & Deschamps, 1975.
Remarks: basidioma resupinate to pileate on buried wood, 2-3 pores/mm; spores cylindric, hyaline, thin-walled, smooth, 8-10.5 (-11) x 3-4 µm. New record for Santa Catarina State.
Distribution: Brazil [Amazonas (Jesus, 1995); Roraima (Jesus, 1996); Paraná (Rajchenberg & Meyer, 1990); São Paulo (Gugliota & Capelari, 1995)]; Argentina (Wright & Deschamps, 1975); Venezuela (Fidalgo, 1968b; Rodriguez et al. 1995); Paraguay (Popov & Wright, 1998).
Material examined: BRAZIL, SC, Ilha de Santa Catarina, Morro Lagoa da Conceição, Gerber, A. L. & Neves, M. A. 899, 900, 25/IV/1996 (FLOR 11413, 11414); PR, Morretes, Meijer, A. 794, 15/III/1987 (BAFC 31282).

*Fomes fasciatus* (Sw.) Cooke
≡ *Polyporus fasciatus* Sw., Syst. Mycol. 1: 373, 1821.
Description in Gilbertson & Ryvarden, 1986.
Remarks: 4-5 pores per mm, spores 12-13.5 x 4 µm similar to those [12-18 (-20) x 4-7 µm] from Gilbertson & Ryvarden (1986). New record for Santa Catarina State.
Distribution: Brazil [Amazonas (Jesus, 1995); Roraima (Jesus, 1996); São Paulo (Gugliota, 1997)]; Venezuela (Fidalgo, 1968b); Paraguay (Popov & Wright, 1998).
Material examined: BRAZIL, SC, Garopaba, Loguercio-Leite, C. & Lentino, Z. 507, 26/II/1994 (FLOR 11317); ibid, ipse, 508, 26/II/1994 (FLOR 11319); Ilha de Santa Catarina, Morro Lagoa Conceição, Neves, M. A., Halmenschlager, M. A. & Degenhardt, R. 555, 556, 22/IX/1994 (FLOR 11074, 11078); ibid, Gerber, A. L. et al. 725, 28/VI/1995 (FLOR 11232); ibid, Gerber, A. L. Loguercio-Leite, C. & Gonçalves, G. 949,

25/VI/1996 (FLOR 11 719); ARGENTINA, Tucumán, Taff Viejo, Guerrero & Bettuci, s/n, 7/II/1965 (BAFC 25231).

*Fomitopsis cupreo-rosea* (Berk.) J. Carranza & Gilb.
≡ *Polyporus cupreo-roseus* Berk., Hook. J. Bot. 8, 1856.
Description in Carranza-Morse. & Gilbertson, 1986.
Remarks: hymenial surface of angular to subdaedaloid pores, 2-3 pores/mm, spores cylindrical to elliptic, hyaline, thin-walled, smooth, 4.5-5.5 x 2.5 μm smaller than those [5.0-7.0 (-8.0) x (2.0) 2.5-3.5 μm] recorded by Carranza-Morse & Gilbertson (1986). New record for Paraná State.
Distribution: Brazil [Amazonas (Ryvarden, 1988); Mato Grosso (Fidalgo, 1968a)]; Venezuela (Fidalgo, 1968b); Colombia and Guiana (Carranza-Morse & Gilbertson, 1986).
Material examined: BRAZIL, PR, Capanema, Basso, J. 989, 04/III/1997 (FLOR 11492).

*Fomitopsis feei* (Fr.) Kreisel
≡ *Polyporus feei* Fr. Linnaea, 5: 518. 1830.
Description in Carranza-Morse. & Gilbertson, 1986.
Remarks: our specimens present 5-6 pores/mm, spores elipsoid to cylindrical, hyaline, thin-walled, smooth, 5-6 x 2.2-2.5 μm, similar data to Carranza-Morse & Gilbertson (1986). New record for Santa Catarina and Paraná States.
Distribution: Brazil [Bahia (Góes-Neto, 1999); Acre (Carranza-Morse & Gilbertson, 1986); Amazonas (Jesus, 1995)]; Argentina (Wright & Deschamps, 1975); Venezuela (Rodriguez et al. 1995); Paraguay (Popov & Wright, 1998).
Material examined: BRAZIL, SC, Ilha de Santa Catarina, Cacupé, Neves, M. A. 1003, 23/VII/1997 (FLOR 11501), ibid, Cruz, C. 19/VIII/1997 (FLOR 11631); ibid, Cacupé, Neves, M. A. s/n, 30/XI/1997 (FLOR 11636); PR, Capanema, Basso, J. 977, -/VIII/1996 (FLOR 11504).

*Henningsia brasiliensis* (Speg.) Speg.
≡ *Polyporus brasiliensis* Speg., Bol. Acad. Nac. Cienc. Cordoba 11(4):436, 1889.
Description in Ginns, 1979.
Remarks: the material presents 7-8 pores per mm, spores subglobose, hyaline, smooth, slightly thick-walled, (3.5-) 4 x 3-3.5 μm, similar to Ginns' data (1979). New record for Santa Catarina Island.
Distribution: Brazil [Bahia (Góes-Neto, 1999); Amazonas (Jesus, 1995); Paraná (Rajchenberg & Meyer, 1990); Santa Catarina - Blumenau (Ginns, 1979)].
Material examined: BRAZIL, SC, Ilha de Santa Catarina, Morro Lagoa da Conceição, 826 (FLOR 11399); PR, Morretes, Meijer s/n, 06/XII/1987 (FLOR 11427).

*Hydnopolyporus fimbriatus* (Fr.) D. A. Reid
≡ *Polyporus fimbriatus* Fr., Linnaea 5: 520, 1830.
Description in Fidalgo, 1963.
Remarks: spores subglobose to broadly elipsoid, thin-walled, smooth, hyaline, 3.4-5 x 3-3.5 μm similar to sizes (3.5-5 x 2.5-3.5 μm) given by Fidalgo (1963). New record for Santa Catarina Island.
Distribution: Brazil [Bahia (Góes-Neto, 1999); Amazonas, Santa Catarina - Blumenau; Mato Grosso; Rio de Janeiro and Rio Grande do Sul (Fidalgo, 1963); São Paulo (Fidalgo, 1963, Gugliota & Capelari, 1995)]; Argentina (Wright & Deschamps, 1976/77, Rajchenberg & Wright, 1987); Colombia

(Fidalgo, 1963); Paraguay (Fidalgo, 1963, Popov & Wright, 1998); Uruguay (Fidalgo, 1963, Gazzano, 1990).

Material examined: BRAZIL, SC, Ilha de Santa Catarina, Lagoinha do Leste, Gerber, Foresti, Althoff 611, 28/III/1995 (FLOR 11070); SP, Ribeirão Preto, Loguercio-Leite s/n, 13/I/1995 (FLOR 11053); ARGENTINA, Santa Fé, Luchatti, s/n, 25/II/1985 (BAFC 30528).

*Laetiporus sulphureus* (Fr.) Murrill

≡ *Polyporus sulphureus* Fr., Syst. mycol. 1: 357. 1821.

Description in Ryvarden & Johansen, 1980.

Remarks: specimes examined show 3-4 pores per mm, spores broadly ellipsoid, hyaline, thin-walled, smooth 4 x 5-6 µm similar to Ryvarden & Johansen's data (1980). New record for Santa Catarina State.

Distribution: Brazil [Bahia (Góes-Neto, 1999)]; Argentina (Wright & Deschamps, 1975); Colombia (Setliff & Ryvarden, 1983).

Material examined: BRAZIL. SC, Ilha de Santa Catarina, Ratones, Cinardi, J., 100, 21/III/1986 (FLOR 10262); ibid, UFSC, Rosenei, L., 640, 19/IV/1995 (FLOR 11127); ibid, Horto Botânico, Souza-Cruz, P. B., 1020 12/XII/1997, (FLOR 11573); ibid, Lagoa do Peri, De Toni, s/n, 01/VIII/1997 (FLOR 11573); ibid, Laguna, Souza, M. A. M. 984, 05/I/1997 (FLOR 11483).

*Lenzites betulina* (L.) Fr.

*Agaricus betulinus* L., Spec. Plant. p. 1176, 1753.

Description in Ryvarden & Gilbertson, 1993.

Remarks: specimens examined present central poroid zone with 1-2 pores/mm; spores cylindrical, often slightly curved, smooth, thin-walled, 4-5 x 2 µm similar to Ryvarden & Gilbertson (1993), who recorded 5-6 x 2-3 µm. First record to Santa Catarina State.

Distribution: cosmopolitan.

Material examinado: BRAZIL, SC, Gramados, Degenhardt, R. 518, 28/VII/1994 (FLOR 11086); ipse, ibde, 530 , 28/VII/1994 (FLOR 11309), ibid, Coqueiros, Bossle, R. C. s/n, 16/II/1985 (FLOR 10080); MEXICO, Xalapa, Vera Cruz, Bondala, V. M. s/n, 18/II/1986 (BAFC 32658).

*Megasporoporia setulosa* (Henn.) Rajchenb.

≡ *Poria setulosa* Henn., Engl. Bot. Jahrb. 28: 321. 1901.

Description in Ryvarden,Wright & Rajchenberg, 1982.

Remarks: specimens examined present 1-2 pores/mm, presence of hyphal pegs (90.1 x 22 µm), spores cylindric, hyaline, thin-walled, smooth, 13-14 (-15.5) x 4.5-5 µm similar to Ryvarden,Wright & Rajchenberg's data (1982). New record for Brazil.

Distribution: Argentina (Ryvarden, Wright & Rajchenberg, 1982); Venezuela (Ryvarden, Wright & Rajchenberg, 1982, Rodriguez et al. 1995)

Material examined: BRAZIL, SC, Ilha de Ratones, Loguercio-Leite, C. Neves, M. A., Cruz, C. Souza, M.A. & Muniz, I. 1036, 24/V/1997 (FLOR 11510)

*Physisporinus sanguinolentus* (Alb. & Schwein.) Pil.

≡ *Boletus sanguinolentus* Alb. & Schwein., Consp. Fung., p.257, 1805.

Description in Gilbertson & Ryvarden, 1987.

Remarks: basidioma white when fresh, waxy, dark brown to black on drying; pores 4-5 per mm, spores subglobose to globose, hyaline, thin-walled, smooth

5-4 x 4 µm, coincides with measurements given by Gilbertson & Ryvarden (1987). New record for Santa Catarina State.
Distribution: Brazil [Bahia (Góes-Neto, 1999)].
Material examined: BRAZIL, SC, Ilha de Santa Catarina, Morro da Lagoa da Conceição, Gerber, A., Loguercio-Leite, C., Foresti, M. A., Althoff, S. & Góes, A. 703, 28/VI/95 (FLOR 11220), CZECHOSLOVAK, Moravia, Vampola, P. 16/VIII/89 (BAFC 32 623).

*Rigidoporus amazonicus* Ryvarden
Description in Ryvarden, 1987.
Remarks: specimens examined show pores 6-8/mm, spores subglobose to globose, hyalines, thick-walled, (3-) 3.2-4 x 3-3.5 µm similar to those from Ryvarden (1987). Second record to the world.
Distribution: Brazil [Amazonas (Ryvarden, 1987)].
Material examined: BRAZIL, SC, Ilhota, Parque do Morro Baú, Degenhardt, R. 1008, 27/X/95 (FLOR 11393).

*Skeletocutis diluta* (Rajchenb.) A .David & Rajchenb.
≡ *Skeletocutis nivea* (Jungh.) Keller var. *diluta* Rajchenb. Mycotaxon 16:505, 1983.
Description in Rajchenberg, 1984.
Remarks: our materials present 4-6 pores/mm; skeletal hyphae dissolving in KOH; spores allantoid, hyaline, thin-walled, smooth, 3-3.5 x 0.5-0.75 µm similar to those given by Rajchenberg (1984). New record for Brazil.
Distribution: Argentina (Rajchenberg, 1983).
Material examined: BRAZIL, SC, Ilha de Santa Catarina, Lagoinha do Leste, Gerber, A., Foresti, R. & Althoff, S., 609, 23/III/1995 (FLOR 11067); ARGENTINA, Misiones, Puerto Esperanza, Rajchenberg s/n, -/III/ 1982 (FLOR 11423).

*Tinctoporellus epimiltinus* (Berk. & Broome) Ryvarden
≡ *Polyporus epimiltinus* Berk. & Broom J. Linn. Soc. 14: 54, 1873.
Description in Ryvarden & Johansen, 1980.
Remarks: our specimens present 5-6 pores/mm; spores subglobose, hyaline, thin-walled, smooth, 4 x 3 µm coinciding to Rajchenberg's data (1984). New record for Santa Catarina State.
Distribution: Brazil [Amazonas (Jesus, 1995)]; Argentina (Rajchenberg, 1984); Venezuela (Fidalgo, 1968b); (Rodriguez et al. 1995); Paraguay (Popov & Wright, 1998).
Material examined: BRAZIL, SC, Ilha de Santa Catarina, Morro da Lagoa da Conceição, Gerber, A., Loguercio-Leite, C., Foresti, M. A., Althoff, S. & Góes, A. 715, 28/VI/95 (FLOR 11620).

*Tyromyces hypocitrinus* (Berk.) Ryvarden
≡ *Polyporus hypocitrinus* Berk. J. Linn. Soc. Bot. 15:50, 1876.
Description in Ryvarden, 1984.
Remarks: our material show pores 2-4/mm, cylindric to allantoid spores, hyaline thin-walled, smooth, 4-6 x 1-1.5 µm similar Ryvarden's data (1984). New record for Santa Catarina State.
Distribution: Brazil [Bahia (Góes-Neto, 1999)].
Material examined: BRAZIL, SC, Ilha de Santa Catarina, Morro da Lagoa da Conceição, Gerber, A., Loguercio-Leite, C., Foresti, M. A., Althoff, S. & Góes, A. 694, 713, 714, 28/VI/95 (FLOR 11244, 11383, 11245).

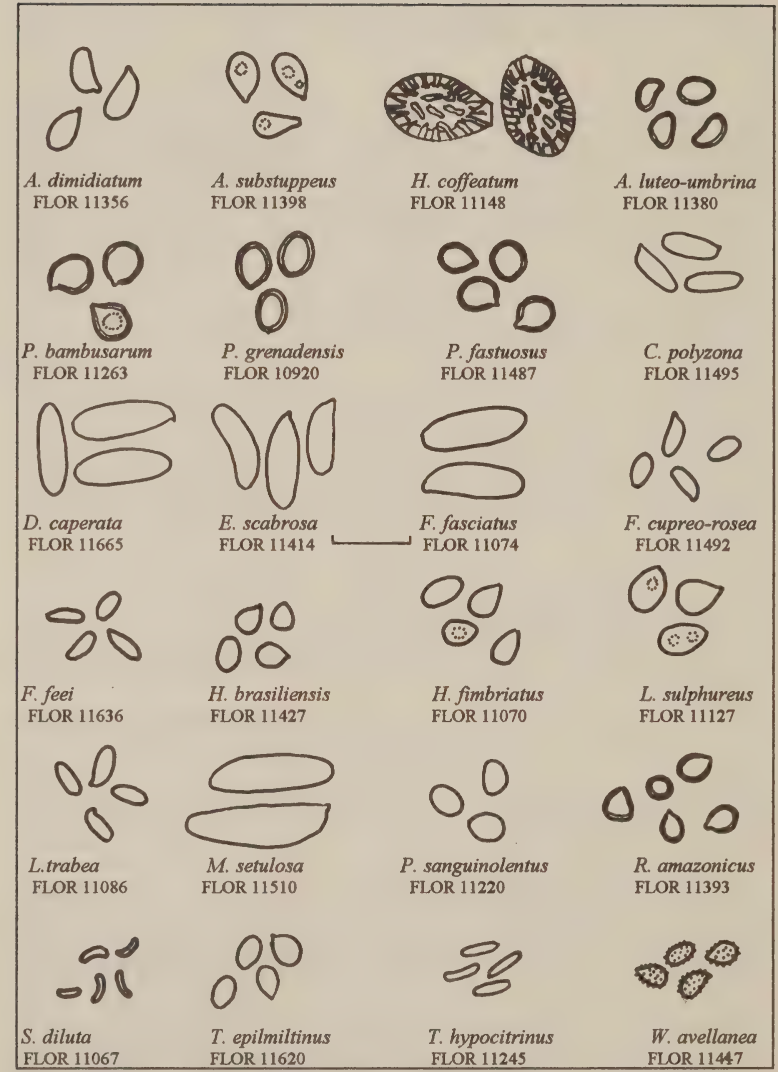

Fig.1. Spores of species presented. Bar=10 μm

## Hericiaceae

*Wrightoporia avellanea* (Bres.) Pouzar
≡ *Poria avellanea* Bres. K. Akad. Wiss. Math. Naturw. Klas. Denk, 83: 14. 1907.
Description in Ryvarden, 1982.
Remarks: our material presents spores, ovoid to ellipsoid, apiculate, slightly thick-walled, asperulate, amyloid, 4-4.5 x 2.5-3 μm bigger than Rajchenberg (1984) who recorded spores 3.1-3.6 x 2.1 μm. New record from Santa Catarina State.
Distribution: Brazil [São Paulo (Ryvarden, 1982)]; Argentina (Rajchenberg, 1984).
Material examined: BRAZIL, SC, Ilha de Santa Catarina, Morro Lagoa conceição, Gerber, Loguercio-Leite, Neves, M. A & Althoff, 846, 21/III/1996 (FLOR 11374),); ibid, ipse, Gerber, A.L., Neves, M. A. & Silva, 934, 31/V/1996 (FLOR 11447) ibid, UCAD - Saco Grande, Gerber, A.L. et al., 1084, 12/08/1997 (FLOR 11577).

## CONCLUSIONS

The present results show that there are numerous wood-rotting species still to be recorded from subtropical areas. 24 new records for Brazil, Paraná and Santa Catarina States and one species, *Rigidoporus amazonicus*, previously registred only in Amazonia State, were found. This study demonstrates the need to collect and study fungi in these subtropical areas around the world to gain a more concise knowledge of the wood-rotting polypores.

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# GLOMUS MINUTUM, A NEW SPECIES IN GLOMALES (ZYGOMYCETES) FROM POLAND

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## Abstract:

An ectocarpic species of the genus *Glomus, G. minutum* (Glomales, Zygomycetes), was discovered in rhizosphere soils of plants colonizing maritime sand dunes of northwestern Poland. *Glomus minutum* is characterized by the formation of small, hyaline spores with a wall consisting of two components: a smooth, permanent outer component and a laminate inner component. None of them stains in Melzer's reagent. Mycorrhizae formed by *G. minutum* in one-species cultures with *Plantago lanceolata* consisted of arbuscules, intra- and extramatrical hyphae.

***Key Words***: Glomales, mycorrhizae, arbuscular fungi, Zygomycetes

## Introduction

Examination of field-collected soils and those resulting from pot trap cultures from maritime sand dunes of northwestern Poland revealed an undescribed species of arbuscular mycorrhizal fungi of the genus *Glomus*. This fungus is described here as *G. minutum*. *Glomus minutum* is characterized by the formation of small, hyaline spores that differ from those formed by other unpigmented members of the genus by wall structure and a clustering habit.

## Materials and Methods

Collection of soil samples, establishment of trap and single-species pot cultures, and growth conditions are as described previously (Błaszkowski and Tadych, 1997). The growth medium of single-species pot cultures was an autoclaved sand of maritime dunes adjacent to Świnoujście (pH-$H_20$ 6.7; 12 and 26 mg $L^{-1}$ P and K, respectively). The host species used in both trap and single-species cultures was *Plantago lanceolata* L. The cultures were harvested after 4-12 mo. Spores were extracted by wet sieving and decanting (Gerdemann and Nicolson, 1963). Mycorrhizas were revealed by the Phillips and Hayman (1970) method, using 0.1% trypan blue.

Morphological properties of spores and their subcellular structures were determined based on at least 100 spores mounted in polyvinyl alcohol/lactic acid/glycerol (PVLG; Koske and Tessier, 1983) and a mixture of PVLG and Melzer's reagent (1:1, v/v). The spores represented all stages of differentiation of the fungus. Terminology of spore structure is that suggested by Walker (1983) and Walker et al. (1998). Spore color was examined under a dissecting microscope on fresh specimens immersed in water. Color names are from Kornerup and Wanscher (1983). Specimens were mounted in PVLG on slides and deposited in the Department of Plant Pathology (DPP), University of Agriculture, Szczecin, Poland, and in the herbarium at Oregon State University (OSC) in Corvallis, Oregon, USA.

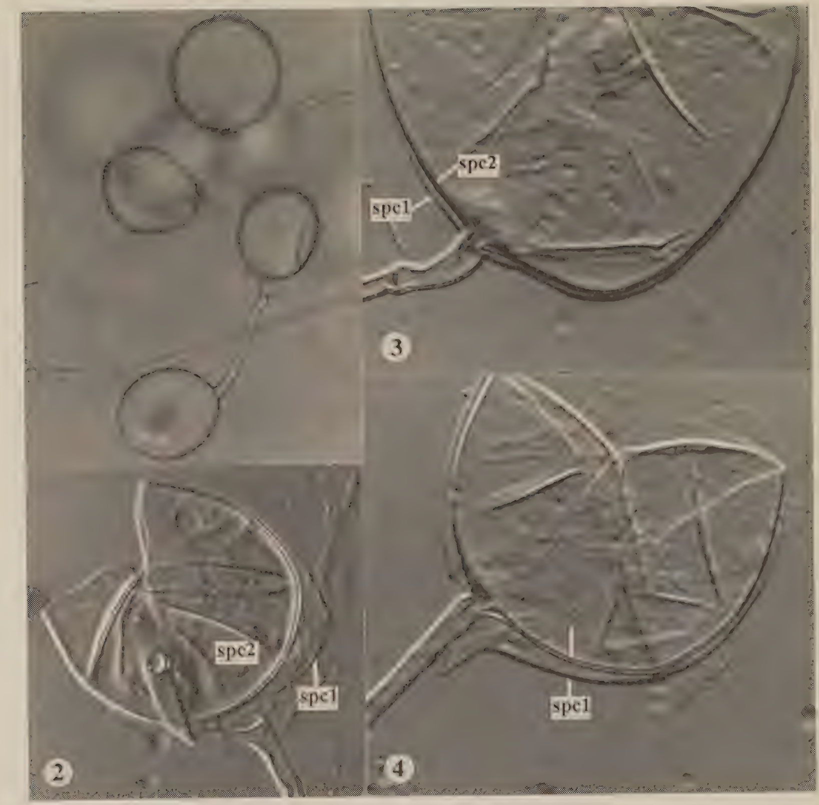
## Descriptions and Discussions

**Glomus minutum** J. Błaszkowski, M. Tadych et T. Madej, sp. nov. Figs. 1-4

*Sporocarpia* ignota. Sporae laxae gregaria, e sporophoris rectic efformatae. Sporophorum nonseptatum vel parce septatum; hyalinum vel pallide aurantiacum; (2.5-)3.0(-3.4) µm latum; pariete (0.5-)0.6(-0.7) µm crasso; rectum. *Sporae* hyalinae; globosae vel subglobosae; (18-)39(-65) µm diam; aliquando ovoideae; 18-30 x 40-80 µm; hypha subtenda solitaria. *Tunica sporae* e startis duobus (strati 1-2); strato ”1” semielastico, hyalino, (0.2-)0.6(-0.7) µm crasso; strato ”2” laminato, hyalino, (0.5-)0.8(-1.2) µm crasso. *Hypha* hyalina; recta vel recurva; cylindrica vel infundibuliforma, raro coliga; (4.2-)5.7(-8.1) µm lata ad basim sporae; pariete hyalino; (0.7-)1.0(-2.7) µm crasso, stratis 1-2 sporae continuo. *Porus* e septo continuo strati 2 sporae efformata. Arbuscular mycorrhizae formans.

HOLOTYPUS. POLAND, Szczecin, infra *Plantago lanceolata* L., 10 Jan. 1999, *Błaszkowski, J.*, *2246* (DPP).

*Sporocarps* unknown. Spores formed singly in the soil or in loose aggregates associated with roots (Fig. 1). *Spores* hyaline; globose to subglobose; (18-)39(-65) µm diam; sometimes ovoid; 18-30 x 40-80 µm; with a single subtending hypha (Figs. 1-4), rarely two. Subcellular structure of spores consists of one wall (Figs. 2-4) with two hyaline components (components 1 and 2). Outermost component 1 smooth, semiflexible, (0.2-)0.6(-0.7) µm thick, tightly adherent to component 2 (Figs. 2-4), flexible in young spores (Fig. 2), semiflexible in mature spores, continuous with two components of subtending hypha (Fig. 4). Component 2 laminate, smooth, (0.5-)0.8(-1.2) µm thick, formed by gradual synthesis of very thin, <0.5 µm thick, laminae in the spore and its subtending hypha. Components 1 and 2 do not react in Melzer's reagent. *Subtending hypha* hyaline; straight or recurved; cylindrical or slightly flared (Figs. 2-4), rarely constricted; (4.2-)5.7(-8.1) µm wide at the spore base. *Wall of subtending hypha* hyaline; (0.7-)1.0(-2.7) µm thick at the spore base; continuous with spore wall components 1-2 (Fig. 4). *Pore* occluded by a septum, 2.0-4.9 µm wide, continuous with the innermost lamina of spore wall component 2. Spore contents of oil droplets.


spc2
spc1
3
spc2
spc1
2
spc1
4

*Collections examined.* HOLOTYPE. POLAND. Szczecin, associated with roots of pot-cultured *P. lanceolata*, 10 Jan. 1999, *Błaszkowski, J., 2246* (DPP); ISOTYPES: *Błaszkowski, J., 2247-2258* (DPP) and two slides at OSC.

*Other materials examined.* POLAND. Spores from trap cultures established based on rhizosphere soils of *Ammophila arenaria* Link, *Corynephorus canescens* (L.) P. B., *Festuca rubra* L., *F. polesica* Zap., *Galium* ? *aparine* L., *Hieracium pilosella* L., *H. umbellatum* L., *Petasites spurius* (Retz.) Rchb. and *Potentilla* ? *anserina* L. colonizing maritime dunes adjacent to Świnoujście (53°55'N, 14°14'E), *Błaszkowski, J., unnumbered collection* (DPP).

*Etymology. minutum*, referring to the small spores formed by the fungus. We wish to dedicate this species to Prof. Dr. Habil. Marian Piech, Department of Biometry, University of Agriculture, Szczecin, a prominent scientist and the former Rector of the Szczecin University of Agriculture.

*Distribution and habitat. Glomus minutum* sporulated abundantly in 14 of the 79 trap cultures containing rhizosphere soils collected on 13 June 1997. The soils came from under dune plants growing near Świnoujście in northwestern Poland (53°55'N, 14°14'E). However, none of the field-collected soils contained spores of this fungus. The lack of spores of *G. minutum* in the field soils may have resulted from three reasons. First, *G. minutum* produces delicate, thin-walled spores that are probably quickly decomposed by soil microorganisms. Parasitic microorganisms may significantly reduce populations of spores of AMF in the field (Lee and Koske 1994). Second, the new fungal species might not have been sporulating at the time of sampling. Seasonal dependence has been observed in sporulation of AMF (Gemma et al. 1989).

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**Figs. 1-4.** *Glomus minutum.* **1.** Cluster of intact spores of different shape, bright field microscopy (BFM), x 513. **2.** Most juvenile spore with a very thin, flexible spore wall component (spc) 1 and a thicker spore wall component 2, differential interference contrast (DIC), x 1428. **3.** Tightly adherent spore wall components (spc) 1 and 2 of a crushed mature spore, DIC x 1428. **4.** Vigorously crushed spore with separated spore wall components (spc) 1 and 2; note the separated subtending hyphal wall components, DIC, x 1428.

Third, *G. minutum* perhaps is a rarely or not sporulating fungus in the field conditions. A high proportion of non-sporulating fungi has been found in different ecosystems (Brundrett et al. 1999; Stutz and Morton 1996).

The plant species associated with *G. minutum* in the field were *A. arenaria*, *C. canescens*, *F. rubra*, *F. polesica*, *G.* ? *aparine*, *H. pilosella*, *H. umbellatum*, *P. spurius* and *P.* ? *anserina*. The arbuscular mycorrhizal fungi accompanying *Glomus minutum* in the field and trap cultures were *Acaulospora trappei* Ames & Linderman, *G. constrictum* Trappe, *G. corymbiforme* Błaszk., *G. lamellosum* Dalpé et al., *G. macrocarpum* Tul. & Tul., *G. microcarpum* Tul. & Tul., *G. pustulatum* Koske et al., undescribed *Glomus* 111, *Glomus* 122, *Glomus* 133, *Scutellospora dipurpurescens* Morton & Koske and *S. persica* (Koske & Walker) Walker & Sander.

The soil chemical properties of the Świnoujście dunes were: pH($H_20$), 3.8-6.7; $NO_3$ (mg $L^{-1}$), 20-72; P, 5-12; K, 2-26; Mg, 10-41; Na, 4-23; Cl, 15-25; KCl, 0.1-0.6; organic C (%), 0.1-1.1 (Błaszkowski, 1995).

*Mycorrhizal associations*. *Glomus minutum* occurred among vesicular-arbuscular mycorrhizal roots of *A. arenaria*, *A. campestris*, *H. arenarium*, *P. spurius*, and *Senecio* sp. The mycorrhizae formed in a single-species culture of this fungal species with *P. lanceolata* were composed of intramatrical hyphae, (3.4-)5.9(-9.1) µm wide, growing parallel to the root axis. These hyphae sometimes formed coils, 12.5-27.5 x 17.5-55.0 µm, short branches swollen at their tip or short, perpendicular branches connected with the neighbouring, parallel hyphae. Arbuscules were numerous and had fine branches that were difficult to see clearly. No vesicles were present in roots of plants even when the cultures were 6 month old. Extramatrical hyphae were 3.2-3.7 µm wide. In 0.1% trypan blue, intramatrical hyphae stained bluish white (22A2), arbuscules bluish white (22A2) to violet white (15A2) and extramatrical hyphae violet white (18A2).

*Discussion*. *Glomus minutum* is unique due to its very small, hyaline spores with a wall consisting of two permanent components, *i. e.*, components present in both the most juvenile and fully mature specimens (Figs. 2-4).

The ontogenetical development of *G. minutum* spores expresses in relatively low increases in size of spores and the thickness of their wall. The changes in wall thickness mainly result from the synthesis of additional laminae in the laminate component 2.

When observed under a stereoscope microscope, spores of *G. minutum* resemble those of *G. diaphanum* Morton & Walker, *G. laccatum* Błaszk., *G. occultum* Walker, *G. spurcum* Pfeiffer et al. and *G. viscosum* T. H. Nicolson (Błaszkowski 1988; Kennedy et al. 1999; Morton 1998; Morton and Walker 1984; Pfeiffer et al. 1996; Walker 1982; Walker et al. 1995). All species form hyaline spores and the largest spores of *G. minutum* attain the lower level of the spore size range of the other species listed above. However, although *G. viscosum* produces spores in loose clusters, *G. minutum* produces them in much tighter clusters that might be thought of as primitive spore bodies.

Examination of *G. minutum* spores under a compound microscope readily facilitates separation of this fungus from other hyaline glomalean species. While the spore wall of *G. minutum* consists of two components (Figs. 2-4), that of *G. diaphanum* and *G. occultum* comprises three. The outermost component of *G. minutum* spore wall is permanent, semiflexible in mature spores and adheres to the innermost laminate component (Figs. 3-4). In contrast, the outermost spore wall component of *G. diaphanum* and *G. occultum* sloughs with age and is usually absent in mature spores. Additionally, this sloughing component is associated with either a middle permanent component (*G. occultum*) or a middle laminate component (*G. diaphanum*). *Glomus minutum* also lacks the innermost semiflexible component of *G. diaphanum* and the innermost permanent component of *G. occultum*.

*Glomus spurcum* produces spores with two permanent wall components, of which, however, the outer one may be darker colored (hyaline to pale yellow-brown) and thicker (0.4-1.2 μm) than that of *G. minutum* remaining hyaline and being not thicker than 0.7 μm. Additionally, the outer component of *G. spurcum* spores easily wrinkles and separates from the neighbouring laminate component (vs. it usually tightly adheres to a laminate component in *G. minutum*), which is much thicker than that of the new fungus described here (1.4-4.2 μm vs. 0.5-1.2 μm thick).

*Glomus viscosum* also has a spore wall structure with two components. However, its outer spore wall component is thicker, more plastic and separates more readily from the laminate component. Additionally, the outer component of *G. minutum* spores does not exude a mucigel-like substance as found in *G. viscosum*.

The features distinguishing *G. minutum* and *G. laccatum* are properties of their spore wall and subtending hypha. The outermost spore wall component of the latter species sloughs with age and usually is absent in mature specimens, and its laminate component is composed of easily separating and thick (ca 0.5-2.2 μm) laminae (vs. inseparable and very thin in *G. minutum*). The subtending hypha of *G. laccatum* compared with that of *G. minutum* is much wider (7.4-12.9 μm vs. 4.2-8.1 μm), and its wall is less compact, because it is formed from loose laminae continuous with those of the laminate spore wall component.

The mycorrhizal colonization of *G. minutum* most resemble those of *G. spurcum* and *G. viscosum* in consisting of only hyphae and arbuscules with no vesicles (Morton 1998; Pfeiffer et al. 1996), which are present in mycorrhizal structures of *G. diaphanum* and *G. occultum* (Morton 1998; Morton and Walker 1984; Walker 1982). However, the mycorrhizae of *G. minutum* stain very lightly in trypan blue, whereas those of *G. spurcum* and *G. viscosum* may be intensively stained. Morphological properties of mycorrhizae of *G. laccatum* are unknown.

## Acknowledgment

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# TAXONOMY AND QUALITATIVE ECOLOGICAL ASPECTS OF POROID HYMENOCHAETALES IN A BRAZILIAN SEASONAL TROPICAL FOREST

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## ABSTRACT

This work comprised a two-year study on diversity and some qualitative ecological aspects of poroid Hymenochaetales in a seasonal tropical forest fragment in Bahia, Brazil. 176 specimens of 10 distinct species were collected: *Aurficaria luteo-umbrina* (Rom.) Reid, *Phylloporia pectinata* (Kl.) Ryv., *Phellinus calcitratus* (Berk. & Curt.) Ryv., *Ph. extensus* (Lév.) Pat., *Ph. gilvus* (Schw.) Pat., *Ph. grenadensis* (Murr). Ryv., *Ph. melleoporus* (Murr.) Ryv., *Ph. membranaceus* Wright & Blumenf., *Ph. palmicola* (Berk. & Curt.) Ryv., *Ph. rimosus* (Berk.) Pilát. Most of species occurred in fallen wood or standing dead trees in partially or completely shadowed sites. 50% of species are typically neotropical. *Phellinus gilvus* was the most frequent species in number of collected specimens as well as in monthly occurrence along sampling period.

**KEY WORDS**: Poroid Hymenochaetales, Diversity, Seasonal Tropical Forest, Bahia, Brazil

## INTRODUCTION

The order Hymenochaetales Oberwinkler is characterized by the presence of setoid structures, euhymenial development, positive xanthochroic reaction and generative hyphae without clamp-connections, comprising two families: Asterostromataceae (Donk) Pouzar and Hymenochaetaceae Imazeki & Toki (Pegler & Young, 1993).

Most of known species of Hymenochaetales belong to the nine poroid genera of Hymenochaetaceae: *Aurificaria*, *Coltricia*, *Coltriciella*, *Cyclomyces*, *Hydnochaete*, *Inonotus*, *Phellinus*, *Phylloporia* and *Pyrrhoderma*. All species are wood-decay fungi, saprobes or facultative parasites of trees and white-rotters, except *Coltricia perennis*, which is ectomycorrizal (Ryvarden, 1991; Hawksworth, 1995).

All the poroid Hymenochaetales genera are widely distributed around the world with either a cosmopolitan or tropical distribution pattern. Even the cosmopolitan genera, however, are better represented in the tropics than in Temperate Zone, with *Inonotus* as the only exception (Ryvarden, 1993). Thus tropical regions, as the Neotropics, are probable centers of diversity of this group and a good place to investigate species richness.

Most of macromycetes biodiversity studies in Neotropics are primarily centered in general surveys and more restricted works emphasizing a specific taxonomic group are quite infrequent. Bondarceva et al. (1992), summarized their extensive taxonomic work in the island of Cuba identifying 45 species of poroid Hymenochaetales. It is the most comprehensive study to date. In Brazil, Sousa (1977, 1980), Loguercio-Leite & Wright (1995) focused their attention to the genus *Phellinus* while Coelho (1994) and Soares & Gugliotta (1998) carried out inventories encompassing Hymenochaetaceae s.l.

Most of the studies previously cited were performed in tropical rain forests where there are no periods of drought along the year. Although much less mycologically explored, seasonal forests submitted to tropical climate constitute an ecosystem, which exhibits intermediate characteristics between vegetation types of wet and dry climatic areas, and could theoretically harbor species usually found in both regions.

In this paper a two-year study on diversity and some qualitative ecological aspects of poroid Hymenochaetales in a tropical seasonal forest fragment in the northeast of Brazil are presented.

## MATERIAL AND METHODS

Fieldwork was carried out along two main tracks, which cross the forest fragment in distinct parts. Monthly collects were made during a period of 2 years (Jul/1995-Jun/1997) comprising three rainy and two dry seasons.

Field information included data about type of substratum, type of wood rot (visual evaluation) and degree of shading of basidiomes. Macroscopic features were analyzed with the aid of a stereomicroscope and freehanded sections were made for the study of microscopic characteristics. Slide preparations were stained with 1% phloxine, 5% KOH, and Melzer's reagent was used to test iodine reaction as in Common (1991). All the specimens collected were deposited in HUEFS with duplicates in ICN. Additional material examined included specimens from BAFC and FLOR. Herbaria designations are those of Holmgren et al. (1990).

## RESULTS AND DISCUSSION

### The studied area

The studied area is a small, but well-preserved fragment of tropical seasonal forest of about 30ha near the edge of "Paraguaçu" river, 200m above sea level. It is situated inside a private farm, "Fazenda Paraíso", in the municipality of Cachoeira, State of Bahia, Brazil – 12°34'S, 38°59'W (fig.1). It is a remaining fragment of a large forested area which was completely covered by water due to the construction of a hydroelectric station in the beginning of 1980s (Bahia, 1984). Ancient legal documents belonging to farm owners confirm the existence of this forest fragment as early as 1863 (Bahia, 1956). Thus it represents the original vegetation cover for that region and, consequently, a good area to search for original mycota.

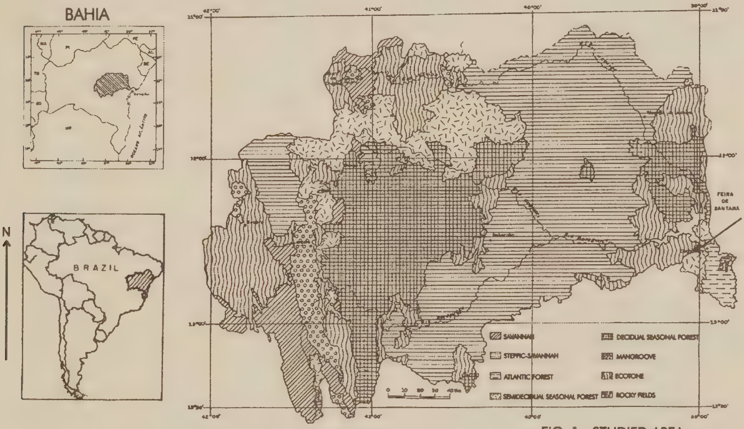

FIG. 1 - STUDIED AREA
(MODIFIED FROM BRASIL, 1990)

The area has a tropical sub-humid climate, with annual temperatures between 22°-25°C and annual rainfall of 800-1200mm. The rainy season is from April to November and the dry one from December to March. The fragment studied comprises a semi-deciduous tropical seasonal forest clearly three-layered: a superior layer composed by medium and big sized trees as *Cavanillesia* sp., *Galesia* sp. *Goniorrachis* sp., *Psidium* sp.; a medium layer, of small sized trees and shrubs; and an inferior one, typically herbaceous. In rainy season the canopy is dense and continuous and the layer next to soil becomes more humid and more protected from direct incidence of solar rays. Conversely in dry season the canopy is sparse and rather open due to the deciduous nature of some canopy trees. Therefore the inferior layer becomes more exposed to direct sunlight and much less humid (Bahia, 1981; Brasil, 1990).

## Taxonomy

### KEY

The key was constructed to emphasize macromorphological and qualitative microscopical characters in order to facilitate the recognition of species in the field or through casual slide examination.

1 Basidiomes resupinate..........................................................................2
1’ Basidiomes pileate..............................................................................4

2 Pores 1-2/mm, hymenial setae 50-80µm long.........*Phellinus palmicola*
2’ Pores 5-7/mm, hymenial setae absent...............................................3

3 Basidiomes annual, beige, very thin, up to 0,1cm thick...........*Phellinus membranaceus*
3’ Basidiomes perennial, brown, more than 0,1cm thick.............*Phellinus melleoporus*

4 Basidiomes annual..............................................................................5
4' Basidiomes perennial..........................................................................6

5 Hymenial setae present, black line in context absent.............*Phellinus gilvus*
5’ Hymenial setae absent, black line in context present............*Aurificaria luteo-umbrina*

6 Upper surface rimose.........................................................*Phellinus rimosus*
6’ Upper surface not rimose.....................................................................7

7 Hymenial setae present.........................................................................8
7’ Hymenial setae absent...........................................................................9

8 Tubes cartilaginous and translucid, spores 4.8-6.0x3.6-5.3µm........................................................................*Phellinus calcitratus*
8’ Tubes not cartilaginous or translucid, spores 3.8-4.8x2.8-4.0µm.......................................................................*Phellinus extensus*

9 Pore surface yellow, spores up to 4.0 long...............*Phylloporia pectinata*
9’ Pore surface brown, spores 4.5-6.0µm long............*Phellinus grenadensis*

## SPECIES

*Aurificaria luteo-umbrina* (Rom.) Reid. Kew Bull. 17:279, 1963. Fig. 15
*Phaeooporus luteo-umbrinus* Rom. Kung. Sv. Vetensk. Akad. Hand. 26 16:27, 1901.
Description in Gilbertson & Ryvarden, 1986:162.
Material studied: HUEFS 41417 (Góes-Neto, 858), ICN 114863 (dupl.); HUEFS 41401 (Góes-Neto, 192), ICN 114851 (dupl.); FLOR 11388.
Remarks: This neotropical species was fairly common in the surveyed area but it is considered to be quite rare (Gilbertson & Ryvarden, 1986). It does not appear in any of all Torrend's papers on polypores of the state of Bahia (Góes-Neto, 1999) nor in any of recent regional taxonomic surveys in Brazil encompassing poroid Hymenochaetales (Cavalcanti, 1976, Sousa, 1977, 1980; Bononi, 1984, 1992; Capelari & Maziero, 1988; Vinha, 1988; Loguercio-Leite, 1990; Rajchenberg & Meijer, 1990,; Silveira & Guerrero, 1991; Coelho, 1994; Fônseca, 1994; Jesus, 1996, Soares & Gugliotta 1998). The thin crust below tomentum, brilliant yellowish context and globose to subglobose olivaceous spores in KOH are decisive for the recognition of this species. It is also recorded for Mexico (Ryvarden & Guzman, 1993).

*Phellinus calcitratus* (Berk. & Curt.) Ryv. Norw. J. Bot. 19:234,1972. Fig. 9
*Polyporus calcitratus* Berk. & Curt. J.Linn.Soc.bot. 10:314, 1868.
Description in Ryvarden & Johansen, 1980:142-143.
Material studied: HUEFS 41453 (Góes-Neto, 304), ICN 114855 (dupl.); BAFC 26552; BAFC 24267.
Remarks: The cartilaginous and translucent tubes are the principal diagnostic feature of this species. The wide, slightly thick-walled, septate, contextual skeletal hyphae (characterizing an intermediate dimitic pattern in context) was also observed by Sousa (1980). Our single collection was on living tree trunk and Coelho (1994) also collected it on living trees. Apparently it is the first citation for the state of Bahia. In Brazil it is also recorded for the state of Rio Grande do Sul (Coelho, 1994) and Amazonian region (Sousa, 1980). For the Neotropical region as a whole, it is recorded for Argentina (Wright & Blumenfeld, 1984), Costa Rica (Carranza-Morse, 1992), Cuba (Bondarceva et al, 1992), Martinique (David & Rajchenberg, 1985) and Venezuela (Dennis, 1970).

*Phellinus extensus* (Lév.) Pat. Ess. Tax. Hym., p.97, 1900. Fig. 10
*Polyporus extensus* Lév. Ann. Sci. Nat. Bot. III, 5:129, 1846.
Description in Ryvarden & Johansen, 1980:158.
Material studied: HUEFS 41425 (Góes-Neto, 423), ICN 114858 (dupl.); HUEFS 41426 (Góes-Neto, 589), ICN 114860 (dupl.); HUEFS 41427 (Góes-Neto, 680); BAFC 31494.
Remarks: All collected specimens were effused-reflexed and with larger pores than usually described in literature (Ryvarden & Johansen, 1980). The short and ventricose setae are diagnostic. It constitutes the first

record for the state of Bahia. In Brazil, Sousa (1980) reported it for Amazon region. It is also recorded for Costa Rica (Carranza-Morse, 1992) Cuba (Bondarceva et al, 1992), Guadeloupe (David & Rajchenberg, 1985) and Venezuela (Dennis, 1970).

*Phellinus gilvus* (Schw.) Pat. Ess. Tax. Hym., p.97, 1900. Fig. 8
*Boletus gilvus* Schw. Schr. Nat. Ges. Leipzig 1:96, 1822.
Description in Gilbertson & Ryvarden, 1987:571-573; var. *licnoides* in Corner, 1991:90-91.
Material studied: HUEFS 41429 (Góes-Neto, 270), ICN 114852 (dupl.); HUEFS 41428 (Góes-Neto, 729); FLOR 11502.
Remarks: *Phellinus gilvus* is one of the most commonly collected fungi in tropical regions. Two distinct varieties were found: (1) characterized by the thin context and distinct zonate and sulcate upper surface of pileus with reddish tint – var. *licnoides*; (2) with thicker context and azonate upper surface, sometimes scrupose, var. *scruposus* (Corner, 1991). Although having been described as perennial and with larger spores (4-5x3-3,5μm), at least in Africa, (Ryvarden & Johansen, 1980), only annual specimens with shorter spores (3,36-4,32x2,00-3,12μm) were collected. Corner (1991) reported small spores (3-5x2-3μm) as did recent regional taxonomic surveys in Brazil [Cavalcanti, 1976, Sousa, 1977, 1980; Loguercio-Leite, 1990; Loguercio-Leite & Wright, 1995, Silveira & Guerrero, 1991; Fônseca, 1994; Soares & Gugliotta 1998]. This species was first recorded for the state of Bahia by Torrend in 1914 as *Polyporus gilvus* (in semi-arid zone) and in 1940 as *Polyporus licnoides*, *Polyporus scruposus* and *Fomes gilvus* (Góes-Neto, 1999). As he followed Lloyd's taxonomic scheme, the last record (as *Fomes gilvus*) suggests the existence of perennial basidiomes of this species in the state of Bahia.

*Phellinus grenadensis* (Murr.) Ryv. Norw. J. Bot. 18:234, 1972. Fig. 11
*Pyropolyporus grenadensis* Murr. North Am. Fl. 9:107, 1908.
Description in Ryvarden & Johansen, 1980:171.
Material studied: HUEFS 41424 (Góes-Neto, 846), ICN 114862 (dupl.); HUEFS 41422 (Góes-Neto, 203); HUEFS 41423 (Góes-Neto, 393).
Remarks: The stratified tubes separated by context layers, the yellowish-brown ellipsoid spores and the somewhat light weight basidiome are rather distinctive features. In Brazil it is cited for the States of Rio Grande do Sul (Sousa, 1977) and São Paulo (Fonseca, 1994). It is the first citation for the State of Bahia. It is also recorded for Costa Rica (Carranza & Ryvarden, 1998), Mexico (Ryvarden & Guzman, 1993) and southern USA (Gilbertson & Ryvarden, 1987).

*Phellinus melleoporus* (Murr.) Ryv. Fig. 13
*Fomitiporella melleopora* Murr. North Am. Flora 9:13, 1907.
Description in Gilbertson & Ryvarden, 1987:584-586
Material studied: HUEFS 41451(Góes-Neto, 406), ICN 114857 (dupl.); BAFC 34580.

Remarks: The ressupinate perennial basidiome, lack of setae and pale golden yellow ellipsoid spores are distinctive features. Spores of collected specimen are slightly smaller than those cited in literature. It constitutes the first record for Brazil. It is recorded for Costa Rica (Carranza-Morse, 1992) and southern USA, Gulf Coast Region (Gilbertson & Ryvarden, 1987).

*Phellinus membranaceus* Wright & Blumenf. Mycotaxon 21:422, 1984. Fig. 14
Description in Wright & Blumenfeld, 1984.
Material studied: HUEFS 41452 (Góes-Neto, 607), ICN 114861 (dupl.); BAFC 29433; BAFC 29862.
Remarks: It differs from *Ph. melleoporus* in having an annual, very thin, beige basidiome firmly adhered to substrate. Apart from the type locality – Iguazu National Park, Missiones, Argentina (Wright & Blumenfeld, 1984), this species is also recorded for Panama (Nuñez & Ryvarden, 1999). It constitutes the first record for Brazil.

*Phellinus palmicola* (Berk. & Curt.) Ryv. Norw. J. Bot, 19:235, 1972. Fig. 7
*Polyporus palmicola* Berk. & Curt. J. Linn. Soc. Bot. 10:317, 1868.
Description in Gilbertson & Ryvarden, 1987:590-591.
Material studied: HUEFS 41454 (Góes-Neto, 538), ICN 114859 (dupl.).
Remarks: Gilbertson & Ryvarden (1987) state this is a neotropical, apparently rare species known only from palms. The single specimen (538), however, was found on a fallen branch of undetermined dicot. The large pores and the straight, subulate and very long hymenial setae are diagnostic features. It is the first record of this species for Brazil. It occurs in Cuba (Bondarceva et al, 1992; Lowe, 1963), Mexico (Lowe, 1966) and the southern United States (Gilbertson & Ryvarden, 1987).

*Phellinus rimosus* (Berk.) Pilát, Ann. Mycol. 38:80, 1940. Fig. 12
*Polyporus rimosus* Berk. Lond. J. Botany 4:54, 1845.
Description in Ryvarden & Johansen, 1980:207-209.
Material studied: HUEFS 41420 (Góes-Neto, 300), ICN 114854 (dupl.); HUEFS 41421 (Góes-Neto, 402), ICN114856 (dupl.); HUEFS 41419 (Góes-Neto, 232).
Remarks: The characteristic rimose upper surface of pileus, perenniality, large brown subglobose to subellipsoid spores and frequent parasitic habit are distinctive features. Young specimens, however, may possess azonate and completely tomentose upper surface and could be confused with *Phellinus nilgheriensis* as it was the case of one of the collected specimens (Góes-Neto, 402). Torrend (1940) reported it for Bahia in semi-arid zone (Steppic-Savannah). In Brazil, it is also cited for the States of Pernambuco (Cavalcanti, 1976), Rio Grande do Sul (Sousa, 1977), São Paulo (Bononi, 1984) and Espírito Santo (Vinha, 1988). For the Neotropical region as a whole, it is recorded for Argentina (Wright & Blumenfeld, 1984), Costa Rica (Carranza-Morse, 1992), Mexico (Ojeda-Lopez, Sandoval, Valenzuela, 1986; Diaz-Barriga, Guevara-Feffer &

Valenzuela, 1988; Pérez-Silva & de la Luz, 1997, Ryvarden & Guzman, 1993) and Paraguay (Popoff & Wright, 1998).

*Phylloporia pectinata* (Kl.) Ryv. Synop. Fung. 5:196, 1991. Fig. 16
*Polyporus pectinatus* Kl. Linnaea 8:486, 1833.
Description in Ryvarden & Johansen, 1980: 195-196.
Material studied: HUEFS 41455 (Góes-Neto, 862), ICN 114864 (dupl.).
Remarks: The parasitic habit as well as the ability to grow on living bushes and trees, frequently on thin branches, are considered remarkable features of *Phylloporia* species (Ryvarden, 1991). Corner (1991) reported that *Phylloporia pectinata* was a typical parasite of understory trees in Malaysia, parasitic on trunks, branches and twigs. The single basidiome of *Phylloporia pectinata*, however, was found on a dead liana. Nevertheless this kind of substrate was completely different from all substrates on which collected specimens of other species had been found. The perenniality, the glancing yellowish pore surface and numerous sulcate zones in upper surface of pileus are distinctive features. The species was recorded for Bahia (as *Fomes pectinatus*) by Torrend (1940) who pointed out that it was commonly found on fallen branches in “Caatinga” (Sttepic-Savanah phytogeographic region). In Brazil, it is also cited in Coelho (1994) for the State of Rio Grande do Sul, in Rajchenberg & Meijer (1990) for the State of Paraná and in Sousa (1980) for the Amazonian region. For the Neotropics as a whole it is recorded for Costa Rica (Carranza-Morse, 1992), Cuba (Bondarceva et al, 1992) and Venezuela (Dennis, 1970; Rodríguez, Burdsall & Volk, 1995).

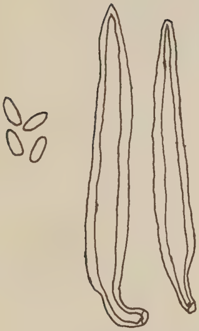

Fig.7 - *Phellinus palmicola*
HUEFS 41454
spores and setae

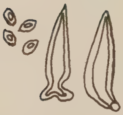

Fig.8 - *Phellinus gilvus*
HUEFS 41429
spores and setae

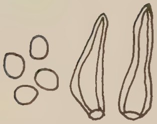

Fig.9 - *Phellinus calcitratus*
HUEFS 41453
spores and setae

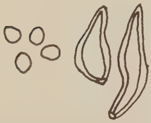

Fig.10 - *Phellinus extensus*
HUEFS 41425
spores and setae

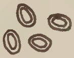

Fig.11 - *Phellinus grendensis*
HUEFS 41424
spores

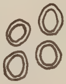

Fig.12 - *Phellinus rimosus*
HUEFS 41420
spores

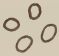

Fig.13 - *Phellinus melleoporus*
HUEFS 41451
spores

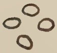

Fig.14 - *Phellinus membranaceus*
HUEFS 41452
spores

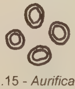

Fig.15 - *Aurificaria luteo-umbrina*
HUEFS 41417
spores

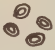

Fig.16 - *Phylloporia pectinata*
HUEFS 41455
spores

## Qualitative Ecological Aspects

A total of 176 specimens were collected along the studied period (fig.2) comprising three distinct genera: *Aurificaria* Reid, *Phellinus* Quél. and *Phyloporia* Murr. The genera *Aurificaria* and *Phylloporia* were both represented by only a single species each: *Aurficaria luteo-umbrina* (Rom.) Reid and *Phylloporia pectinata* (Kl.) Ryv. respectively. In contrast, eight different *Phellinus* species were identified: *Ph. calcitratus, Ph. extensus, Ph. gilvus, Ph. grenadensis, Ph. melleoporus*, *Ph. membranaceus*, *Ph. palmicola* and *Ph. rimosus.*

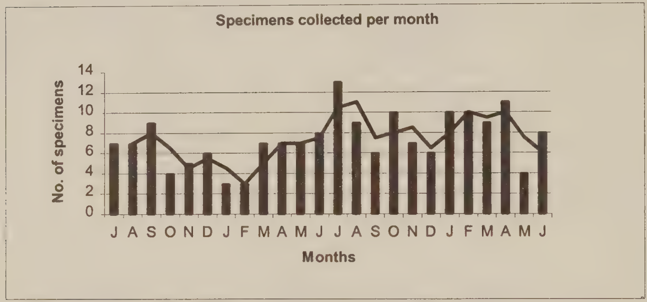

Fig.2: Variation of number of collected specimens per month

*Ph. gilvus* was, by far, the commonest species with up to 77% of total collected specimens. *Phy. pectinata*, *Ph. calcitratus*, *Ph. melleoporus*, *Ph. membranaceus* and *Ph. palmicola*, however, were collected only once. The following table correlates species with total number of collections (fig.3).

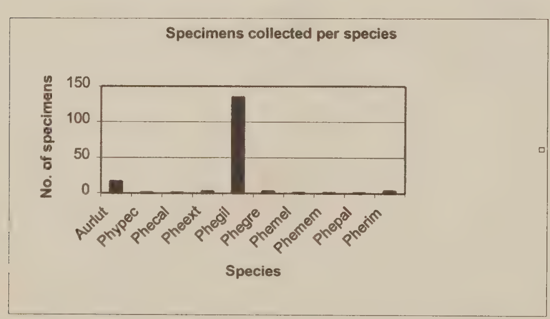

Fig.3: Number of collected specimens per species

Although *Ph. gilvus* was extremely common, 83% of collected specimens were sterile. This situation sharply contrasts with other species where spores were always found in tube layer(s) of basidiomes, irrespective of annual or perennial condition.

Spore producing basidiomes of at least one of the aforementioned ten poroid hymenochaetaceus species were found every month in the two-year field work except for a short exceedingly dry period of three months (Dec/95, Jan-Fev/96) (fig 4).

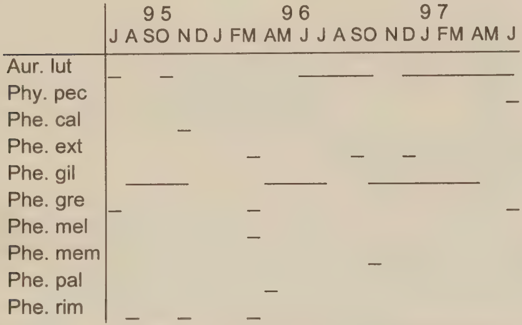

Fig.4: Occurrence of spore-producing basidiomes

All species are typical white-rotting fungi and occurred in fallen wood, dead trees still upright and living ones (fig 5). Most of species were always found in dead wood irrespective if they had been in fallen trunks and branches or dead upright trees. Only three species were found in living trees: *Phellinus rimosus, Phellinus calcitratus* and *Aurificaria luteo-umbrina*.

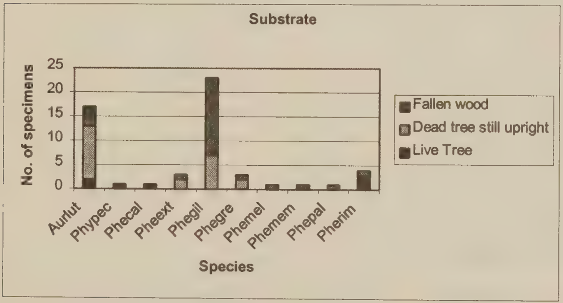

Fig.5: Type of substrate and species

Most *Phellinus rimosus* specimens occurred in living trees of Caesalpiniaceae (Leguminosae) or Myrtaceae as could be expected since this species is known to be parasitic on genera of several woody plants, especially Leguminosae (Kotlaba & Pouzar, 1978; Gilbertson & Ryvarden, 1987; Bernicchia, 1990). In contrast, only 12% of total collected specimens of *Aurificaria luteo-umbrina* were found in living dicots suggesting that they might be occurring in dead tissues of living tree trunks.

Basidiomes of all species were frequently found either in open or shadowed sites, thus submitted to different light conditions (fig.6). Most of *Phellinus gilvus* collected basidiomes occurred in open areas, especially clearings in the forest fragment and usually in great number.

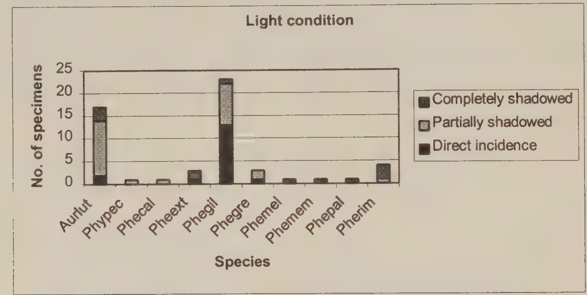

Fig.6: Different light conditions and collected specimens

## CONCLUSIONS

The forest fragment studied revealed at least one species in ten that is typical of drier areas (*Phellinus rimosus*) suggesting that species of either humid or dry regions may actually occur in seasonal forests.

In the studied area 50% of the species exhibit a neotropical distribution pattern (Table I).

Table I - Distribution patterns of identified species

| Species | Pantropical | Neotropical |
|---|---|---|
| *Aurificaria luteo-umbrina* | | x |
| *Phylloporia pectinata* | x | |
| *Phellinus calcitratus* | | x |
| *Phellinus extensus* | x | |
| *Phellinus gilvus* | x | |
| *Phellinus grenadensis* | x | |
| *Phellinus melleoporus* | | x |
| *Phellinus membranaceus* | | x |
| *Phellinus palmicola* | | x |
| *Phellinus rimosus* | x | |

Although the number of identified species in the area is quite small, the high percentage of neotropical species suggests that Neotropics are actually a center of diversity of poroid Hymenochaetales.

A recent broader taxonomy survey (macromycetes in general) in a more humid forest area in the same State of Bahia revealed three species of poroid Hymenochaetales (*Phellinus piptadeniae*, *Phellinus sancti-georgii*, *Phellinus wahlbergii*) that were not found in the seasonal forest fragment of this study (Góes-Neto, 1999). The former two species are also neotropical.

Local rainfall differences do affect the species composition of poroid Hymenochaetales in Bahian tropical forest environments but more comparative ecological and taxonomic studies must be carried out, especially including the semi-arid region, to a better understanding of this aspect.

## ACNOWLEDGEMENTS

We wish to thank the owners of 'Fazenda Paraíso', João de Góes Canna Brasil and Altamira Góes Canna Brasil, for their invaluable collaboration, Flávio Luiz and Lica de Sousa, for line drawings and map, Dr. Leif Ryvarden, for confirmations and/or determinations and, especially, Dr. James Ginns, for his critical review.

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# MYXOMYCETES IN THE FORESTS AND WOODLANDS OF WESTERN OREGON

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## ABSTRACT

This study examined the zonal distribution of myxomycetes in the Pacific Northwest, with special emphasis on the forests and woodlands of western Oregon. Our material included specimens of at least 77 species of myxomycetes, many of which occur mainly on wood or bark. Most specimens were obtained from moist chamber cultures. *Elaeomyxa cerifera* is reported as new for North America north of Mexico; this species, one species of *Cribraria*, and two species of *Licea* are described in detail. According to our data, species richness and productivity of myxomycetes are highest in the mixed forests and woodlands of inland valleys. Wet coniferous forests at both ends of an elevational gradient exhibit lower values of species richness and productivity. The main pattern observed is that of increasing myxomycete diversity with increasing heterogeneity in substrate types, especially in relation to increasing availability of angiosperm bark and wood among potential substrates for myxomycetes.

**Keywords**
Myxomycota, vegetation zones, *Elaeomyxa*

## INTRODUCTION

Myxomycetes are common and often abundant organisms in forests and woodlands throughout the world. Most live in decaying plant material and tend to associate with litter, coarse woody debris, or the bark of living trees. Relatively little is known about their habitat relationships, both among different types of substrates within individual forests and among different types of forests on regional scales.

The objective of this study was to identify major patterns in the distribution of myxomycetes in the forests and woodlands of the Pacific Northwest and to suggest factors that may be important in influencing such patterns. The area studied supports a wide variety of forest vegetation types reflecting great diversity in local climate, topography, and soil conditions. In western Oregon and Washington, these range from wet coniferous forests at low elevations west of the Cascade Crest, with mixed forests and broadleaf woodlands in interior valleys, through montane and subalpine coniferous forests at high elevations in the Cascades, to dry coniferous forests and woodlands at lower elevations east of the Cascade Crest. The Klamath-Siskiyou region of SW Oregon and NW California supports mixed evergreen forests with a distinctive, sparse vegetation on serpentine soils.

The myxomycete flora of the Pacific Northwest, though presumably quite diverse, has attracted relatively little attention. The first comprehensive study of myxomycetes in Oregon is that of Peck and Gilbert (1932). They dealt with the northern third of the Willamette Valley, the Oregon Coast Ranges north of Lincoln County, and the western slopes of the Cascade Range south to what is now the Three Sisters wilderness. However, the authors remarked that within this area whole counties were not studied at all. Peck and Gilbert reported the results of field collections made during twenty years in NW Oregon and listed 194 species including nine new to science. Some of the species described as new have since been reduced to synonyms of previously described species (Martin and Alexopoulos 1969). Curtis (1970) studied myxomycetes in Crater Lake National Park which is located in southeastern Oregon. He listed 43 species, 16 of which were not reported by Peck and Gilbert. Kowalski (1968) described a new species of *Diderma* (*D. subcaerulaeum* Kowalski) from the same location.

## MATERIAL AND METHODS

### Field work

Myxomycetes for this study were collected by the second author during his stay at the Department of Botany and Plant Pathology, Oregon State University, from August 1997 to July 1998. Most specimens were acquired during field studies of pin-lichens (Caliciales, Ascomycota) and non-lichenised members of the family Mycocaliciaceae (Ascomycota) in western Oregon, western Washington, and NW California. Some additional specimens were kindly provided by Eric B. Peterson (OSU) and Maggie Rogers (Portland, Oregon). In addition to myxomycete fruiting bodies, substrate samples were collected for preparation of moist chamber cultures.

Field collections were made from many different types of forests and woodlands along a complex environmental gradient over mountainous terrain. Over 60 localities were surveyed for site-level species inventories, with a primary goal to maximise species capture in the limited amount of time allotted (1–6 h per site). Many calicioid fungi are associated with old, damaged trees and, as a result, most myxomycete specimens were acquired from old-growth forests. Specimens and substrate samples were collected mainly from diversity hotspots for calicioid fungi; these typically include the basal trunks of large living trees, snags, and hardwood twigs. In order to improve myxomycete species capture, logs in an advanced stage of decay, litter, and/or dung also were sampled at most sites (Table 1).

Myxomycete specimens were collected from 41 localities, and many of these including several sites. A few localities were in western Washington, SW Oregon, or NW California, but the majority were situated along a broad west-east transect across NW Oregon (Fig 1). The collecting localities are briefly described below. They have been organised into seven groups (A–G) corresponding to major vegetation zones or zone complexes, the delimitation of which essentially follows those of Franklin and Dyrness (1988).

Table 1. Substrate spectra and other explanatory variables for the different forest zones. Variables are as follows: Elev, average elevation of collecting sites; Conif, percentage of conifer wood and bark in substrate samples; Broad, percentage of angiosperm wood and bark in substrate samples; Litter, percentage of detritus in substrate samples; Dung, percentage of dung in substrate samples; pH, average pH of moist chamber cultures; Sp Rich, species richness of myxomycetes.

| Forest Zone | Elev m | Conif % | Broad % | Litter % | Dung % | pH | Sp Rich |
|---|---|---|---|---|---|---|---|
| *Picea sitchensis* zone (A) | 121 | 74.2 | 4.3 | 17.2 | 4.3 | 4.6 | 19 |
| *Tsuga heterophylla* zone (B) | 429 | 70.7 | 7.8 | 18.6 | 2.9 | 4.3 | 20 |
| Valley foothills (C1) | 177 | 58.1 | 17.3 | 20.7 | 3.9 | 4.9 | 41 |
| Valley bottomland (C2) | 149 | 15.9 | 58.6 | 22.3 | 3.2 | 5.7 | 29 |
| Cascades, W-slope (D) | 1110 | 97.4 | 2.6 | 0 | 0 | 4.4 | 11 |
| Cascades, E-slope (E) | 1298 | 64.1 | 6.2 | 29.7 | 0 | 4.5 | 14 |
| Subalpine forests (F) | 1743 | 84.5 | 2.2 | 13.3 | 0 | 4.5 | 8 |
| Klamath, Oregon (G1) | 475 | 85.7 | 14.3 | 0 | 0 | 4 | 7 |
| Klamath, California (G2) | 1160 | 83.9 | 12.9 | 3.2 | 0 | 4 | 6 |

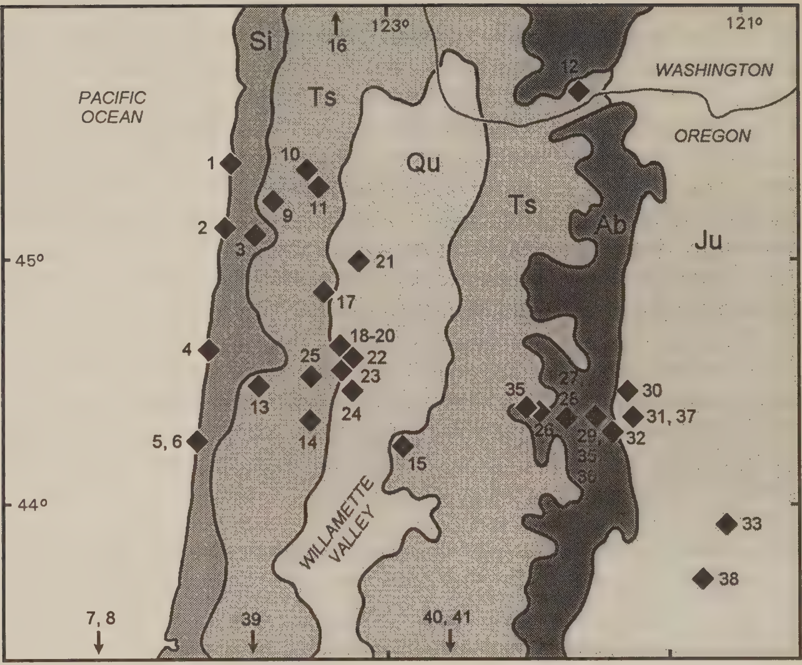


Figure 1. Generalised vegetation map of NW Oregon and SW Washington (based on Franklin & Dyrness 1988), showing collection localities (1–41). Zonal maps fail to present local elevational variation in vegetation very accurately. As such, in the present study we used a more detailed classification of forest vegetation as a framework for data collection and discussion. A rough comparison of the zonal vegetation map and our system is as follows: Si, *Picea sitchensis* zone (zone A in our system); Ts, *Tsuga heterophylla* zone (mainly zone B in our system); Qu, forests and grasslands of the Willamette Valley (zone C in our system); Ab, montane and alpine vegetation of the Cascades (zones D–F in our system); Ju, forests and steppes east of the Cascade Crest (zones E–F in our system).

**A. *Picea sitchensis* Zone.** – Temperate coniferous forests extending as a narrow zone from Alaska to south-western Oregon. The zone is usually only a few kilometres wide, and in central Oregon its elevational range extends from sea level to 100–600 m, depending on local topography. The climate is maritime and wet. Annual precipitation averages 2000 to 3000 millimetres, with frequent fogs giving additional moisture during the relatively drier summer months. *Picea sitchensis* characterises the zone, although in many places *Pseudotsuga menziesii* and *Tsuga heterophylla* dominate. *Alnus rubra* characterises riparian sites and *Thuja plicata* is common in swampy habitats. *Pinus contorta* dominates stabilised dune communities.

**1.** OREGON. Tillamook Co.: Cape Lookout State Park. 45°20.58′N, 123°59.24′W. Elev. 90 m. 19 May 1998. **2a–d**. Tillamook Co.: Cascade Head. **2a**: Cascade Head Experimental Forest

Headquarters. 45°03′N, 123°53′W. Elev. 55 m. 21 Oct. 1997. **2b**: Upper Cascade Head Conservance Trail. 45°03′N, 124°00′W. Elev. 365 m. 21 Oct. 1997. **2c**: Forestry Road 1861. 45°03.2′N, 123°54.3′W. Elev. 215 m. 22 Oct. 1997. **2d**: Hart's Cove Trail. 45°04.3′N, 124°00.3′W. Elev. 120 m. 22 Oct. 1997. **3**. Lincoln Co.: H. B. Van Duzer Forest Corridor Wayside. 45°02.25′N, 123°48.63′W. Elev. ca. 150 m. 12 May 1998. **4**. Lincoln Co.: Mike Miller Educational Forest. 44°36.3′N, 124°03.2′W. Elev. 30 m. 10 Dec 1997. **5a–b**. Lane Co.: Cummins Creek Wilderness. 44°16′N, 124°06′W. 11 Mar. 1998. **5a**: Gwynn Creek. Elev. 40–80 m. **5b**: Outlet of Gwynn Creek. Elev. 15 m. **6a–d**. Lane Co.: Cummins Creek Wilderness. 44°16′N, 124°06′W; 19 Mar. 1998. **6a**: Cummins Creek. Elev. 90–120 m. **6b**: Cummins Creek. Elev. 40–70 m. **5c–d**: Cummins Creek Trailhead. Elev. 80 m. **7a–b**. Curry Co.: Humbug Mountain State Park. 5 Apr. 1998. **7a**: 42°41.1′N, 124°26.1′W. Elev. 50–100 m. **7b**: 42°40.9′N, 124°26.4′W. Elev. 120–500 m. **8**. Curry Co.: Cape Sebastian. 42°20.3′N, 124°25.1′W. Elev. 70–120 m. 2 Mar. 1998.

**B. *Tsuga heterophylla* Zone**. – Temperate coniferous forests extending from British Columbia to northern California and mantling the Coast Range and lower western slopes of the Cascades Range. Elevational range in Oregon is from 150 to 1000 m. The climate is mild and moist, with greater moisture and temperature extremes than in the coastal *Picea sitchensis* zone. Annual precipitation averages 1500 to 3000 millimetres and occurs mainly during the winter. Although named for the potential climax species, *Tsuga heterophylla*, the forests are often dominated by the seral *Pseudotsuga menziesii*. *Alnus rubra*, *Acer macrophyllum*, and *Thuja plicata* characterise riparian and swampy sites. *Abies amabilis* is important at higher elevations, and particularly in the south, *Pinus ponderosa* and *Calocedrus decurrens* also occur in this zone.

**9**. OREGON. Tillamook Co.: Little Nestucca River. 45°07.6′N, 123°50.1′W. Elev. 170–250 m. 17 Apr. 1998. **10a–b**. Tillamook Co.: Nestucca River Area of Critical Environmental Concern. 45°19,0′N, 123°27,8′W. 6 May 1998. **10a**: Elev. 460–500 m. **10b**: Elev. 460 m. **11**. Tillamook Co.: Sheridan Peak Research Natural Area. 45°17′N, 123°28′W. Elev. 750–850 m. 7 Mar. 1998. **12a–c**. WASHINGTON. Skamania Co.: Wind River. **12a**: Wind River Experimental Forest. 44°48.5′N, 121°57.2′W. Elev. 320 m. 25 Oct. 1997. **12b–c**: Thorton T. Munger Research Natural Area. **12b**: Whistle Punk Trail. 45°49′N, 121°58′W. Elev. ca. 350 m. 15 Nov. 1997 and 5 June 1998. **12c**: Wind River Canopy Crane Research Facility. 45°49.14′N, 121°57.07′W. Elev. 355 m. 5 June 1998. OREGON. **13**. Lincoln Co.: Flynn Creek Research Natural Area. 44°32.3′N, 123°51.2′W. Elev. 170–250 m. 17 Apr. 1998. **14**. Benton Co.: Alsea Falls. 44°19.7′N, 123°30.0′W. Elev. 260 m. 10 Aug. 1997. **15a–b**. Linn Co.: Horse Rock Ridge Research Natural Area. 44°18′N, 122°52′W. 25 May 1998. **15a**. Elev. 760 m. **15b**. Elev. 740 m. **16**. WASHINGTON. Thurston Co.: Bald Hill Natural Area. 46°49'N, 122°26'W. Elev. ca. 200 m. 26 Mar. 1998.

**C. Willamette Valley Forest-Grassland Complex**. – Temperate forests, woodlands, and grasslands occupying the bottomland and adjoining slopes of the Willamette Valley. Enclosed by the Coast Range to the west and the Cascade Range to the east. Elevational range is from 60 to 300 m., a relatively warm and dry region in the rain shadow of the Coast Range. Summers are warm and dry, winters mild and wet. Annual precipitation averages 800 to 1200 millimetres. Many forests and woodlands in the Willamette Valley are under extensive human influence.

**C1. Foothill Forests**. – Temperate coniferous forests and mixed forests on the foothills of the Willamette Valley. Temperatures are somewhat lower and precipitation is higher than at the bottom of the valley. *Pseudotsuga menziesii* is dominant, but *Abies grandis* and *Acer macrophyllum* also are common. *Tsuga heterophylla* is excluded from all but the peripheries of the region where foothill forests grade into the *Tsuga heterophylla* Zone.

**17**. OREGON. Polk Co.: Little Sinks Research Natural Area. 44°50.29′N, 123°26.39′W. Elev. 200 m. 2 Feb. 1998. **18a–c**. Benton Co.: McDonald Research Forest, near Peavy Arboretum. 44°39.9′N, 123°14.6′W. **18a–b**: Between Cronemiller Lake and Powder House. **18a**: Elev. 200 m. 28 Nov. 1997. **18b**: Elev. 180 m. 5 Dec. 1997. **18c**: Forest Discovery Trail W of Arboretum. Elev. ca 150 m. 5 Dec. 1997. **19**: Benton Co.: McDonald Research Forest, Sulphur Springs. 44°38.47′N, 123°18.79′W. Elev. 180–185 m. 19 Dec. 1997 and 12 May 1998. **20**: Benton Co.: McDonald Research Forest, Oak Creek. 44°36,2′N, 123°20,1′W. Elev. ca. 150 m. 22 June 1998.

**C2. Bottomland forests and woodlands**. – Temperate forests and woodlands on the bottom of the Willamette Valley. Open woodlands are dominated by *Quercus garrayana*, with *Toxicodendron diversiloba* and various grasses in the understory. *Acer macrophyllum* is prominent on mesic sites, while *Fraxinus latifolia* and *Populus trichocarpa* characterise riparian forests. Many broadleaf forests and woodlands are being invaded by *Pseudotsuga menziesii* and *Abies grandis*.

**21**. OREGON. Polk Co.: Baskett Slough National Wildlife Refuge. 44°58.1′N, 123°15.5′W. Elev. 120 m. 10 Nov. 1997. **22a–c**: Benton Co.: Chip Ross Park. 44°36.4′N, 123°16.9′W. **22a**: Elev. 175–200 m. 5 Dec. 1997. **22b**: Elev. 240–250 m. 18 Nov., 25 Nov., 5 Dec. 1997. **22c**: 44°36.4′N, 123°16.9′W. Elev. 235 m. 5 Dec. 1997. **23**. Benton Co.: Willamette Park. 44°32.4′N, 123°14.9′W. Elev. 65 m. 24 Nov. 1997. **24a–c**. Benton Co.: William L. Finley National Wildlife Refuge. **24a–b**: Mill Hill. 5 Oct. 1997. **24a**: 44°24′N, 123°20.9′W. Elev. 130 m. **24b**: 44°23.9′N, 123°20.7′W. Elev. 130 m. **24c**: Woodpecker Loop. 44°35′N, 123°20.1′W. Elev. 100 m. 7 Feb. 1998.

**D. Montane Forests West of the Cascade Crest**. – Upper orotemperate and lower oroboreal coniferous forests on the western slopes of the Cascade Range extending from British Columbia to central Oregon (*Abies amabilis* Zone). Similar forests also are found on high peaks of the northern Oregon Coast Ranges. Elevational range in Oregon is from 900 to 1500 m. The climate is relatively mild and wet, with a large proportion of precipitation falling as snow. Annual precipitation averages 2000 to 3000 millimetres. Forest composition varies widely, depending on stand age and locale. Typical tree species include *Abies amabilis*, *Abies procera*, *Pseudotsuga menziesii*, and *Tsuga heterophylla*. The major climax species is *A. amabilis*. Areas of volcanic ash support stands of *Pinus contorta*. Dense thickets of *Alnus sinuata* characterize sites with heavy snow accumulations or abundant seepage water. Broadleaf trees are uncommon, but many stands have well-developed shrub layers. Lush herbaceous understories characterize mesic and hygric sites.

**25**. OREGON. Benton Co.: Mary's Peak. 44°30.4′N, 123°33.3′W. Elev. 1050–1150 m. 12 Aug. 1997. **26**. Linn Co.: Hackleman Grove. 44°23.89′N, 122°05.60′W. Elev. 1100 m. 23 June 1998. **27**. Linn Co.: Near Santiam Junction. 44°25′N, 122°00′W. Elev. 1000 m. 27 Aug. 1997. **28a–b**. Linn Co. Santiam Junction. 44°26.3′N, 121°59.9′W. 27 Aug. 1997. **28a**: Elev. 1150 m. **28b**: Elev. 1200 m.

**E. Montane Forests and Woodlands East of the Cascade Crest**. – Temperate and lower oroboreal forests clothing the eastern slopes of the Cascade Range and extending to interior mountains. A complex array of forest formations characterized by *Pinus ponderosa* (*Abies grandis* and *Pinus ponderosa* Zones). Also open woodlands typified by *Juniperus occidentalis*, mixing with shrub-steppe communities at the lower timberline (*Juniperus occidentalis* Zone). Many midslope positions east of the Cascade Crest support coniferous stands characterized by high diversity in tree species, particularly in seral stands. The elevational range in Oregon is from 1100 to 1500 m. The climate is relatively mild and moist with most of the precipitation falling as snow. Annual precipitation averages 600 to 1500 millimetres. Major tree species include *Abies grandis*, *Pseudotsuga menziesii*, *Pinus ponderosa*, *Pinus contorta*, and *Larix occidentalis*, with additional species occurring in more limited numbers or localized areas. *Pinus ponderosa* forests occupy a narrow band on the eastern flanks of the Cascade Range as well as large areas in the interior mountains of the Pacific Northwest. Elevational range in central Oregon is about 900 to 1500 m, depending on locale. The climate is characterized by a short growing season and minimal summer rains. Annual precipitation averages 350 to 750 millimetres, and much of it falls as snow. Climax *P. ponderosa* stands are typically quite open, with few other tree species, except *Juniperus occidentalis* in xeric localities and *Populus tremuloides* in riparian stands. *Abies grandis*, *Pseudotsuga menziesii*, *Larix occidentalis*, *Pinus contorta*, and *Pinus monticola* are common associates in seral stands. *Juniperus occidentalis* woodlands represent the most xeric tree-dominated formation in the Pacific Northwest. Summers are very hot and dry. Annual precipitation averages 200 to 350 millimetres with almost all rain falling during the winter months. *Juniperus occidentalis* is typically the only tree species present along with a sparse undergrowth of *Artemisia tridentata*, various grasses, and herbs.

**29a–b**. OREGON. Elliot R. Corbett II Memorial State Park. 9 June 1998. **29a**: Deschutes Co.: Between Cub Lake and Cache Mt. 44°23.14′N, 121°47.93′W. Elev. ca. 1300 m. **29b**: Jefferson Co.: Island Lake. 44°24.32′N, 121°48.30′W. Elev. ca. 1200 m. **30**. Jefferson Co.: Candle Creek. 44°34.46′N, 121°39.67′W. Elev. ca. 900 m. 18 June 1998. **31**. Jefferson Co.: Black Butte. 44°24.07′N, 121°38.81′W. Elev. ca. 1680 m. 18 June 1998. **32**. Deschutes Co.: Windy Point.

44°17.5′N, 121°45.8′W. Elev. 1460 m. 10 May 1998. **33**: Deschutes Co.: Horse Ridge. 43°56.35′N, 121°03.69′W. Elev. ca. 1250 m. 19 June 1998.

**F. Subalpine Forests**. – Oroboreal forests along the crest of the Cascade Range and on high mountains east of the Crest (*Tsuga mertensiana* and *Abies lasiocarpa* Zones). Lower elevational boundary is usually 1500 m or more. The climate is cool and wet. Annual precipitation averages 1500 to 3000 millimetres. Cool summers, cold winters, and the development of thick winter snow-packs are characteristic. Forest composition varies widely, depending on locale. The major climax species is *Tsuga mertensiana*, with *Abies lasiocarpa*, *Abies amabilis*, and *Pinus contorta* being important associates. In more continental areas, *A. lasiocarpa* is the major climax species. *Chamaecyparis nootkatensis*, *Pinus monticola*, and *Picea engelmannii* also occur in this zone. At high elevations, the continuous forest breaks into a mosaic of forest patches interspersed with shrubby or herbaceous meadow communities.

**34**. OREGON. Linn Co.: Echo Basin. 44°24.83′N, 122°05.64′W. Elev. 1300 m. 23 June 1998. **35**. Jefferson Co.: Santiam Pass. 44°25.7′N, 121°50.5′W. Elev. 1500 m. 27 Aug. 1997. **36**. Jefferson Co.: Hayrick Butte. 44°24.29′N, 121°51.94′W. Elev. 1550 m. 9 June 1998. **37a–b**: Jefferson Co.: Black Butte. 44°24.00′N, 121°38.50′W. 18 June 1998. **37a**: Elev. ca. 1750 m. **37b**: Elev. 1960 m. **38a–c**. Deschutes Co.: Newberry Crater. 19 June 1998. – **38a**: Between East Lake and The Dome. 43°62.85′N, 121°11.09′W. Elev. ca. 2150 m. **38b**: Paulina Lake. 43°63.52′N, 121°16.63′W. Elev. 1935 m. **38c**: Paulina Creek Falls. 43°62.77′N, 121°16.83′W. Elev. ca. 1800 m.

**G. Klamath forests**. – Temperate coniferous forests in the Klamath region of SW Oregon and NW California. The region is known for diverse topography and geology, and it has an interesting floristic history. Climatically, it lies in a transition zone between the wet temperate climate of the western Pacific Northwest and a drier Mediterranean climate in the south. Summers are hot and dry, winters mild and moist. At higher elevations, much of the annual precipitation falls as snow. Many forests in the region are northern extensions of Sierra montane forests. *Pseudotsuga menziesii*, *Pinus ponderosa*, and *Calocedrus decurrens* dominate the upper canopy of many stands, with sclerophyllous trees and shrubs, such as *Arbutus menziesii*, *Quercus chrysolepis*, and *Lithocarpus densiflorus* in the lower canopy and shrub layer. Serpentine soils bear a distinctive flora and their forests are sparse and stunted in comparison with those on non-serpentine soils. *Pinus jeffreyi* is the dominant tree on xeric sites, but *Calocedrus decurrens* is also common. A mixture of conifers, including *Pseudotsuga menziesii* and *Chamaecyparis lawsoniana*, occur on mesic and hygric sites, but these forests also tend to be sparse and xerophytic in appearance. The understory is usually dominated by sclerophyllous shrubs. The herb coverage is low but rich in species.

**39a–d**. OREGON. Josephine Co.: Rough and Ready Creek watershed. 28 Feb. 1998. **39a**: Rough and Ready Creek Forest Wayside State Park. 42°05.4' N, 123°41.2' W. Elev. ca. 400 m. **39b–c**: Near crossing of Forest Service Road 442. **39b**: 42°05' N, 123°44' W. Elev. 500 m. **39c**: 42°05.1' N, 123°45.4' W. Elev. ca. 520 m. **39d**: 42°05' N, 123°44'W. Elev. ca. 480 m. **40a–b**. CALIFORNIA. Trinity Co.: Scott Mountain. 17 Sep. 1997. **40a**: 41°16.38′N, 122°62.13′W. Elev. ca. 1000 m. **40b**: 41°15.81′N, 122°61.67′W. Elev. ca. 800 m. **41a–b**. Trinity Co.: Bear Gulch Trail. 40°53′N, 123°01,3′W. 18 Sep. 1997. **41a**: Elev. 1200 m. **41b**. Elev. 1650 m. **41c**: Trinity Co.: Ripstein Gulch. 40°52,8′N, 123°01,67′W. Elev. 880 m.

### Laboratory work

Moist chamber cultures were prepared from 780 substrate samples. Two hundred ninety-five cultures were prepared with bark, 267 with decayed or living wood, 188 with detritus or litter, and 30 with dung (Tables 1 & 2). The biological material was placed in sterile Petri dishes (9.5 cm diam.) on a single layer of filter paper (in some cases the culture material was fairly scanty). Before wetting, the cultures were inspected under a dissecting microscope for pre-existing slime moulds. The dishes were then filled with distilled water adjusted to pH 7.0 with KOH. After 48 hours, the pH of the water was measured with pH strips (Merck Universalindikator), and some of the excess water was poured off. The cultures were incubated at normal room conditions (ca 20°C) in diffuse day light and examined under a dissecting microscope every second or third day. The first 159 cultures were incubated for four weeks, after which they were dried for two weeks. They then were rewetted and incubated for another four weeks. If representatives of a myxomycete species appeared twice in the same Petri dish, they were counted as one specimen. Six hundred and twenty-eight cultures were incubated for only one four week period, after which they were allowed to dry slowly for one week. When developing myxomycetes emerged, the pH was measured once more and the lid of the Petri dish was left slightly ajar.

The myxomycete specimens were identified according to methods of classical herbarium taxonomy, including examination under dissecting and compound microscopes. SEM micrographs were obtained with a Jeol JSEM-820 scanning electron microscope in the Institute of Biotechnology, Electron Microscopy unit, University of Helsinki. All specimens are deposited at the Botanical Museum of the University of Helsinki (H). Some of these are scanty and exist only as permanent slides.

## RESULTS

### Species list

A total of 396 myxomycete specimens was identified, 201 of which developed in moist chamber cultures (Table 2). The biota included 77 species in 30 genera, most of them representing taxa with wide, more or less cosmopolitan distributions. *Elaeomyxa cerifera* is reported for the first time

Table 2. Data on moist chamber cultures from the different forest zones. Attributes are as follows: Samples, substrate samples studied under the microscope; Obs, myxomycete observations (incl. plasmodia and taxa not identified to species); MCC, moist chamber cultures; MCC %, percentage of all substrate samples cultivated; MCC Sp, sporulating myxomycete specimens in cultures; MCC Pl, cultures with plasmodia failing to sporulate; Prod A, productivity (%) of cultures based on sporulating myxomycetes; and Prod B, productivity (%) of cultures based on all myxomycete observations.

| Forest zone | Samples | Obs | MCC | MCC % | MCC Sp | MCC Pl | Prod A | Prod B |
|---|---|---|---|---|---|---|---|---|
| *Picea sitchensis* zone (A) | 232 | 65 | 163 | 70.6 | 21 | 11 | 12.9 | 19.6 |
| *Tsuga heterophylla* zone (B) | 242 | 63 | 126 | 52.1 | 4 | 17 | 3.2 | 16.7 |
| Valley foothills (C1) | 179 | 145 | 138 | 77.1 | 58 | 16 | 42.0 | 53.6 |
| Valley bottomland (C2) | 157 | 102 | 77 | 49.0 | 52 | 11 | 67.5 | 81.8 |
| Cascades, W-slope (D) | 77 | 38 | 67 | 87.0 | 19 | 4 | 28.4 | 34.3 |
| Cascades, E-slope (E) | 128 | 39 | 108 | 84.4 | 20 | 3 | 18.5 | 21.3 |
| Subalpine forests (F) | 90 | 18 | 67 | 74.4 | 11 | 0 | 16.4 | 16.4 |
| Klamath, Oregon (G1) | 35 | 13 | 23 | 65.7 | 12 | 0 | 52.2 | 52.2 |
| Klamath, California (G2) | 31 | 15 | 11 | 35.5 | 4 | 1 | 36.4 | 45.4 |
| Total | 1171 | 498 | 780 | 66.6 | 201 | 63 | 25.8 | 33.8 |

from North America north of Mexico. This rare species is described and illustrated, based on the material collected from Oregon. One unusual species of *Cribraria* that developed in moist chamber culture is described with illustrations and two species of *Licea* are also described in detail. Some 30 myxomycete species are newly reported for Oregon.

In the species list below, the arrangement of families and genera follows that of Nannenga-Bremekamp (1991); species within each genus are listed alphabetically. An asterisk before a species name indicates that the taxon was previously reported from Oregon by Peck & Gilbert (1932), by Martin & Alexopoulos (1969), or by Curtis (1970). Numbers in bold after species names refer to collecting sites. Most collection numbers are those of the second author. In voucher specimens, all of these collection numbers are preceded by *JRL* (with L indicating a myxomycete specimen, as ascomycetes also were obtained from most field samples). *EP* in front of an collection number refers to Eric B. Peterson. Substrate data for epiphytic and lignicolous myxomycetes are usually given in abbreviated form (tree species/substrate). Abbreviations of tree species are: ABGR, *Abies grandis*; ABLA, *Abies lasiocarpa*; ABPR, *Abies procera*; ABSP, *Abies* sp.; ACMA, *Acer macrophyllum*; ALRU, *Alnus rubra*; ARME, *Arbutus menziesii*; CADE, *Calocedrus decurrens*; CHLA, *Chamaecyparis lawsoniana*; CHNO, *Chamaecyparis nootkatensis*, COSP, conifer sp.; FRLA, *Fraxinus latifolia*; JUOC, *Juniperus occidentalis*; PISI, *Picea sitchensis*; PIAL, *Pinus albicaulis*; PICO, *Pinus contorta*; PIJE, *Pinus jeffreyi*; PIMO, *Pinus monticola*; PIPO, *Pinus ponderosa*; POTR, *Populus trichocarpa*; PSME, *Pseudotsuga menziesii*; QUCH, *Quercus chrysolepis*; QUGA, *Quercus garrayana*; SARA, *Sambucus racemosa*; TABE, *Taxus brevifolia*; THPL, *Thuja plicata*; TSHE, *Tsuga heterophylla*, and TSME, *Tsuga mertensiana*. The substrates of epiphytic and lignicolous myxomycetes were usually pooled either into "bark" or "wood", which are abbreviated b and w, respectively. Finally, "mc" indicates that the myxomycete specimen developed in moist chamber culture. Collection dates can be found in the list of collecting localities.

### **Family Ceratiomyxaceae** Schroet.

****Ceratiomyxa fruticulosa*** (F. Muell.) T. Macbr. **10b:** *98311B*, COSP/w. **11:** *98137Fb*, COSP/w. **13:** *98286H*, COSP/w.

### **Family Echinosteliaceae** Rostaf.

***Echinostelium minutum*** de Bary **24c:** *98030a*, QUGA/w, mc (mixed with *Arcyria cinerea*). **39b:** *98057b*, PIJE/w, mc (mixed with *Comatricha nigra*).

### **Family Liceaceae** Rostaf.

***Licea belmontiana*** Nann.-Bremek. **25:** *97012*, ABPR/w; *97006b*, *97018b*, ABPR/b (the latter specimen mixed with *Licea minima*). **26:** *98660*, TSHE/w; *98669*, ABSP/w. **28a:** *97030*, COSP/w. **28b:** *97036*, PSME/w. **30:** *98A6*, *98A9*, *98A10*, ground litter under conifers. **32:** *98321b*, COSP/w (mixed with immature *Comatricha* sp.); *98323*, PIPO/w. **33:** *98D7*, ground litter. **34:** *98641a*, ABPR/w (mixed with immature *Comatricha* sp.); *98648*, ABSP/w. **38c:** *98635*, TSME/w. – All specimens developed in moist chamber culture. One specimen, *98D7*, is identified as *L. belmontiana* with some hesitation, since the (slightly immature) sporangia are not only dark brown, but also medium brown, and the spore mass is not dark brown, but brown, also in transmitted light somewhat paler than in the other specimens recorded.

***Licea biforis*** Morgan **19:** *97340c*, TABE/w, mc (mixed with *Arcyria cinerea* and *Didymium squamulosum*).

***Licea*** cf. ***castanea*** G. Lister OREGON. Lane Co.: **Eagles Rest:** Old-growth *Pseudotsuga menziesii* forest on ridge. 43°85′N, 122°75′W. Elev. 820 m. *EP12-3Ab* (mixed with *Comatricha pulchella*); *EP12-3Bb; EP12-3Cb; EP12-3Da.* – These fairly scanty specimens seem to be closest to *L. castanea*. The sessile sporangia are pale brown, olivaceous brown or fairly dark brown, 0.1–0.5 mm diam., the margin of the peridial lobes usually bear two rows of warts. The spores are pale ochraceous yellow in mass, pale yellow in transmitted light, thick-walled with a clear thinner and paler area, 10–12 µm in diam. The spores, however, are not totally smooth, but very minutely warted. According to Nannenga-Bremekamp (1991) and Neubert, Nowotny & Baumann (1993) the spores of *L. castanea* are smooth, according to Martin & Alexopoulos (1969) smooth to nearly smooth.

**Other material studied.** *Licea castanea* G. Lister. USA: IOWA, Iowa City, 1 Nov. 1951. A. L. Welden 6567, det. N.E. Nannenga-Bremekamp. State University of Iowa Myxomycete Collection; BPI 826092, (BPI). Finland: Kimito, 2 Jan. 1999. P-G. Wikström 5414, 3 Mar. 1999 . P-G. Wikström 5418–5423 (H).

***Licea kleistobolus*** G. W. Martin **17:** *98028*, COSP/w, mc (mixed with immature *Comatricha* sp.).

***Licea minima*** Fr. **8:** *98103*, PSME/w, mc. **11:** *98117b*, PSME/w. **12a:** *97165Db*, PSME/b (mixed with immature *Stemonitis* sp.). **17:** *98009, 98014, 98027,* COSP/w, mc. **22b:** *97203b*, QUGA/b and w (mixed with *Comatricha laxa* and *Stemonitis* sp.). **24c:** *98035*, COSP/w, mc. **25:** *97014b, 97022b,* ABPR/w, mc (the latter specimen mixed with *Licea* sp. 2); *97018a*, ABPR/b, mc (mixed with *Licea belmontiana*). **32:** *98314*, PIPO/w, mc. – Since Peck and Gilbert did not use the moist chamber culture technique when collecting myxomycetes in Oregon, they report this species to be rare, occurring in the Willamette Valley. Curtis (1970) record on collection on decayed wood.

***Licea pygmaea*** (Meylan) Ing **18c:** *97265Bb*, COSP/bark of exposed roots (mixed with *Physarum viride*). **32:** *98322*, ABSP/w, mc.

**Other material studied**. Switzerland: Lectotype of *Licea pusilla* Schrad. var. *pygmaea* Meylan, La Gittaz, Canton Vaud, Oct. 1930, C. Meylan, (LAU).

***Licea*** cf. ***testudinacea*** Nann.-Bremek. **25:** *97017*, ABPR/b, mc. – The spores of *L. testudinacea* have a very conspicuous pale germination area (Nannenga-Bremekamp 1991). In the Oregon specimen, the spores are thick-walled with a paler area, but this is not very conspicuous. The sporangia, however, are somewhat immature, enclosing some large and irregular-shaped spores.

**Other material studied**. Netherlands: Doorweth, 14 July 1965. Leg. et. det. Nannenga-Bremekamp 5995. The University of Texas Myxomycete Collection, UTMC-636; BPI 826505, (BPI).

***Licea*** **sp. 1**. (Figs 2–6). **24c:** *98039b, 98041b*, QUGA/b, mc (the first specimen grew mixed with *Cribraria violacea*, the latter with *Macbrideola martinii*). – Sporangia scattered, sessile, hemisphaerical to subglobose, occasionally somewhat narrowed at the base, cream-coloured when the peridium is crusted with refuse matter, dark, shiny and somewhat iridescent when refuse matter is absent; 95–120 µm in diam. Peridium thin, pale brown to translucent, inner side with scattered papillae, outer part crusted with cream-coloured refuse matter to nearly bare, then shiny, iridescent, appearing dark since the dark spore mass shines through; with no preformed lines of dehiscence, upper part dehiscing away and leaving basal cup, basal part occasionally thickened and brown. Spores dark shiny brown in mass, brown with a reddish tint in transmitted light, globose, thick-walled with large thin and pale area; 10–$\underline{12.3}$–13(–15) µm in diam.

In sporangia with only scanty deposits of or no refuse matter on the peridium, there is some resemblance to *Licea hydrargyra* Nann.-Bremek., Lakh. & Chopra. This species has sporangia "looking like drops of mercury", but the peridium is smooth on both sides, and the spores are black in mass, olivaceous in transmitted light, (12.5–)13–14(–15) in diam. (Lakhanpal, Nannenga-Bremekamp & Chopra 1990). *Licea perexigua* T. E. Brooks & H. W. Keller also may macroscopically resemble our material. In this species, however, the inner surface of the peridium is closely and minutely papillose, and the spores are smaller (8.5–10.5 µm), and pale olivaceous yellow in transmitted light (Keller & Brooks 1977). We also studied the holotype of *L. pseudoconica* Brooks & Keller, and two additional specimens of this species kindly loaned by Dr. Harold Keller (Central Missouri State University). The globose to globoid sporangia of *L. pseudoconica* appear more or less conic because there is a conic mass of whitish refuse matter on top of them. In our material, the sporangia are comparatively large, 95–120 µm in diam., the pale mass of refuse matter is sometimes aggregated at the top of the sporangia, but usually the lateral parts are also covered with it (Fig. 3). Some sporangia lack the peridial refuse matter, they appear dark grey brown and iridescent because the glossy, dark brown spore mass shines through (Fig. 2). The inner side of the peridium bears scattered papilla and is not closely papillose as in *L. pseudoconica*. Furthermore, the spores are slightly larger, and their colour is pale brown (Figs 5–6). The spores of *L. pseudoconica* are olive-brown, 9.5–11(–13) in diam. (Keller & Brooks 1977).

**Other material studied**. USA.: KENTUCKY. Holotype of *Licea pseudoconica*, Cumberland Co., 5 Aug. 1965, T. E. Brooks 2844, fide H. W. Keller; BPI 737651, (BPI). Kentucky Co., 18 July 1962, T. E. Brooks 2515 (Private herbarium of H. W. Keller). FLORIDA. Citrus Co., 4 Sep. 1977, H. W. Keller 2237 2515 (Private herbarium of H. W. Keller). USA.: ARKANSAS. Holotype of *Licea perexigua,* Crawford Co. T. E. Brooks 2747, (BPI).

***Licea*** sp. 2. **25:** *97014c*, ABPR/b, mc (mixed with *Licea minima*). – Sporangium sessile, ochraceous brown, globose, 0.3 mm in diam. Peridium thin, ochraceous, slightly wrinkled, dehiscing irregularly, ochraceous yellow in transmitted light, inner side pale yellow, warted. Spores ochraceous yellow in mass, pale yellow in transmitted light, in clusters, subglobose to turbinate, minutely spinulose on the exposed area, the remainder smooth, thick-walled, (8.5–)9–$\underline{9.5}$–12 µm in diam. – The specimen is very scanty, only one sporangium existing as a permanent slide. It closely approaches *Licea syncarpon* Nann.-Bremek., but this species has a dark brown spore mass, making the sporangia appear dark brown. Some *Perichaena* species may occasionally lack a capillitium. However, the only *Perichaena* species with clustered spores, *Perichaena syncarpon* T. E. Brooks, has a double peridium (the outer layer of which is cartilaginous), a bright yellow spore mass, and the spores are globose to subglobose and more uniformly covered with spines.

**Other material studied**. USA: KANSAS. Isotype of *Perichaena syncarpon*, Geary Co., T. E. Brooks 920, 2 July 1945, BPI 830634, (BPI).

### Family Reticulariaceae Rostaf.

*****Tubifera ferruginosa*** (Batsch) J. F. Gmel. **2d:** *97151*, PISI/w. **18b:** *97285A*, COSP/w. **30:** *98562a*, PIPO/w. **40a:** *97057*, PSME/w.

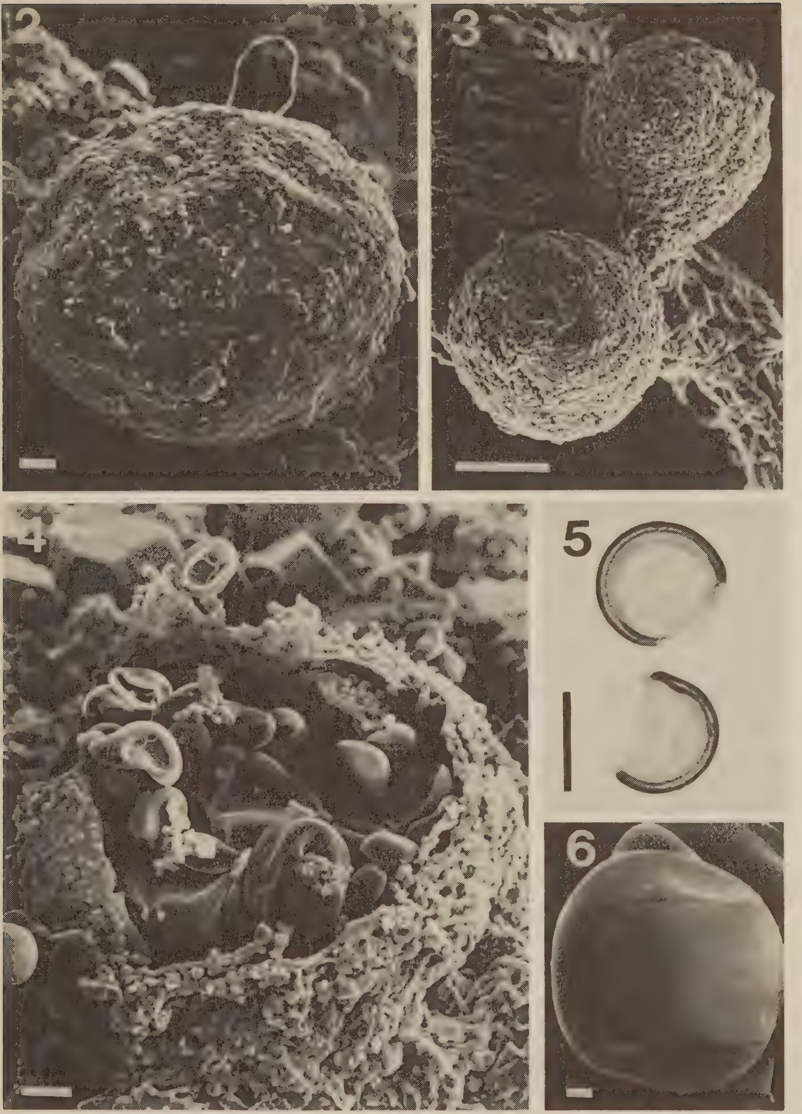
***Lycogala conicum*** Pers. OREGON. Linn Co.: **Keel Flats:** Riparian old-growth *Pseudotsuga menziesii–Tsuga heterophylla* forest. 44°51´N, 122°65´W. Elev. 490 m. *EP10-3*.

*****Lycogala epidendrum*** (L.) Fr. **2b:** *97127*, ALRU/w. **18b:**, *97246Aa, 97247*, COSP/w (the first specimen mixed with *Cribraria vulgaris*); *97251A*, ACMA/w. **18c:** *97265A*, COSP/wood of exposed roots.

***Reticularia intermedia*** Nann.-Bremek. **33:** *98598*, JUOC/w.

### Family Cribrariaceae Rostaf.

*****Cribraria minutissima*** Schwein. **4:** *97309I*, COSP/w, mc. – A scanty specimen consisting of only five sporangia. Peck and Gilbert (1932) reported this



Figures 2–6. *Licea* sp. 1 (*JRL98039b*). Figs 2–4, 6 scanning electron micrographs (SEMs). Fig. 2. Hemisphaerical sporangium thinly covered with refuse material. Fig. 3. Two subglobose sporangia. Fig. 4. Dehiscent sporangium showing smooth spores. Fig. 5. Thick-walled, smooth spores in light microscope. Fig. 6. Smooth spore. Scale bars: Figs 2, 4, 5 = 10 µm, Fig. 3 = 50 µm, Fig. 6 = 1 µm.

species to be rare in NW Oregon. They found it during spring on well rotten wood in the Willamette Valley.

***Cribraria montana*** Nann.-Bremek. **5a:** *98161*, PISI/b, mc. **5b:** *98160b*, PISI/w, mc (mixed with *Arcyria pomiformis* and *Dianema corticatum*).

**Other material studied**. Sweden: Uppland. Alunda, 31 May 1962, R. Santesson 14498 (det. as *C. microcarpa*), rev. det. Nannenga-Bremekamp 1973. Öland. Bödda par., 12 July 1962, R. Santesson 14643 (det. as *Cribraria microcarpa*), rev. Nannenga-Bremekamp 1973. Härjedalen. Tännas par., 12 Aug. 1962, R. Santesson 14891b (det. as *C. microcarpa*), rev. Nannenga-Bremekamp 1973. Hälsingland. Ängersjö par., 17 Aug. 1962, R. Santesson 14999 (det. as *Cribraria minutissima*), rev. Nannenga-Bremekamp 1973.

***Cribraria violacea*** Rex **18c:** *97273A*, detritus under epiphytic bryophytes on basal trunk of living *Pseudotsuga menziesii*, mc; *97280b*, ACMA/w (mixed with *Didymium squamulosum*). **22a:** *97291Ab, 97291C, 97291E*, leafy ground litter, (the first specimen mixed with *Arcyria cinerea*). **22b:** *97294B*, detritus under epiphytic bryophytes on branches of living *Quercus garrayana*. **24c:** *98034*, QUGA/b and w; *98039a*, QUGA/b (mixed with *Licea* sp. 1); *98043b*, FRLA/b (mixed with *Perichaena chrysosperma*). – All specimens developed in moist chamber culture.

***Cribraria vulgaris*** Schrad. **18b:** *97245*, ACMA/w; *97246Ab*, COSP/w (mixed with *Lycogala epidendrum*); *97246C, 97246E, 97246F*, COSP/w. **18c:** *97269Cb, 97285C*, COSP/w. – Peck and Gilbert (1932) described *Cribraria oregana* H. C. Gilbert as a new species. It has brown sporangia, 0.2–0.4 mm in diameter, 0.5–1.2 mm high, the nodes of the peridial net are large in comparison to the size of the sporangium, and the spores are 8–9.5 µm. Peck and Gilbert (1932) found this taxon several times on well rotted Douglas fir logs in the Willamette Valley. Martin & Alexopoulos (1969) describe the sporangia of *C. oregana* to be up to 1 mm high, dull orange-brown to dark brown (different from the colour of *C. vulgaris*). The spore diameter given by them is (6.5–)7–8.5(–9) µm. In Neubert *et al.* (1993) the colour is said to be orange-brown to light chestnut brown, and the spore diameter is given as 7–8 µm. Nannenga-Bremekamp (1991) includes *Cribraria oregana* as a variety of *C. vulgaris* (Nannenga-Bremekamp & Lado 1985). The material identified here as *C. vulgaris* has sporangia up to 2.5 mm high (mostly about 1.5–2 mm), up to 0.7 mm in diameter, the colour is ochraceous to hazel brown, and the spores are 6–$\underline{7}$–8 µm in diam.

**Other material studied.** Finland: Merimasku, 20 June 1860, P. A. Karsten. Kirkkonummi, 31 July, Marja Härkönen 1301. Ikaalinen, 17 July 1979, Marja Härkönen 1697, 1707. Viljakkala, 19 July 1979, Marja Härkönen 1690. Konnevesi, 19 Aug. 1986, Marja Härkönen 3377 (*C. vulgaris* var. *oregana*). Joroinen, 28 July 1914, T. J. Hintikka. (H).

***Cribraria*** sp. (Figs 7–10). **6a:** *98192D*, ground litter under conifers, mc. – Sporangia scattered, stalked, erect or nodding, delicate, globose, pale dusky brown; total height about 1 mm, 0.08-0.12 mm in diam. Stalk long, slim, pale brown, tapered and paler (yellowish) towards the apex, yellowish brown in transmitted light, the basal part filled with refuse matter. Peridial cup only a small disc, radiating stellately into the threads of the peridial net, filled with pale dusky brown dictydine granules, 1.5–3 µm in diam.; peridial net lax, regular, meshes mostly four-angular, knots small, irregular in shape, not thickened, with dictydine granules similar as those in the basal disc; connecting threads without granules, stiff, about 1 µm in diam. Spores dusky brown in mass, pale rosy brown in transmitted light, globose, minutely spiny, some of the spines unite into short ridges, 6–6.5–7(–7.5) µm in diam. – The specimen is scanty, consisting of about 15 sporangia. Under a dissecting microscope, it resembles a rose-coloured *Echinostelium minutum*, since the peridial net and nodes are not very conspicuous (Figs 7–8).

### **Family Dianemaceae** T. Macbr.

****Calomyxa metallica*** (Berk.) Nieuwl. **12a:** *97165Ca*, PSME/b (mixed with *Physarum viride*).

****Dianema corticatum*** Lister **5b:** *98160c*, PISI/w, mc (mixed with *Arcyria pomiformis* and *Cribraria microcarpa*). – According to Martin and Alexopoulos (1969), this species is rare in North America, but Peck and Gilbert (1932) report it to be common during winter rains in the Willamette Valley and in the Cascades.

### **Family Arcyriaceae** A. & G. Lister

***Perichaena chrysosperma*** (Curr.) Lister **24c:** *98043a*, FRLA/b, mc (mixed with *Cribraria violacea*).

***Perichaena depressa*** Lib. **33:** *98D4, 98D6*, ground litter, mc.

***Arcyria affinis*** Rostaf. emend. Nann.-Bremek. **11:** *98137Cb, 98137Db*, COSP/w. **18b:** *97251C*, ACMA/w. **18c:** *97266A*, COSP/bark of exposed roots. **22b:** *97200*, QUGA/b.

****Arcyria cinerea*** (Bull.) Pers. **18c:** *97267Ab, 97268Aa, 97269Db*, ground litter (mixed with *Didymium squamulosum*). **19:** *97340b*, TABE/w (mixed with *Didymium squamulosum* and *Licea biforis*). **20:** *98430*, FRLA/b. **22a:** *97291Aa*, leafy ground litter (mixed with *Cribraria violacea*). **22b:** *97286Cb*, leafy ground litter (mixed with *Didymium squamulosum*). **23:** *97209a*, POTR/b (mixed with immature *Didymium* sp., possibly *D. squamulosum*); *97211*, ACMA/w. **24c:** *98030b*, QUGA/w (mixed with *Echinostelium minutum*). – All specimens developed in moist chamber culture. Peck and Gilbert (1932) mention *A. cinerea* to be common but never abundant. They report it from the Willamette Valley.

**∗*Arcyria denudata*** (L.) Wettst. **17:** *98025*, ACMA/w. **18b:** *97250*, ACMA/w.

**∗*Arcyria ferruginea*** Sauter **11:** *98137Bb*, COSP/w.

**∗*Arcyria incarnata*** (Pers.) Pers. **15b:** *98490*, TSHE/b. **18b:** *97259*, bark and soil on exposed roots of living *Pseudotsuga menziesii*. **19:** *97337*, TABE/w; *97343*, ACMA/w. **22b:** *97223*, QUGA/w. **24c:** *98037a*, QUGA/w (mixed with *Arcyria pomiformis* and *Comatricha nigra*); *98042*, FRLA/w. – All specimens developed in moist chamber culture.

**∗*Arcyria nutans*** (Bull.) Grev. (syn. *Arcyria obvelata* (Oeder) Onsberg) **22b:** *97202B*, QUGA/w. **41b:** *97073*, QUCH/w.

**∗*Arcyria pomiformis*** (Leers) Rostaf. **5b:** *98159, 98160a*, PISI/w (the later specimen mixed with *Cribraria microcarpa* and *Dianema corticatum*). **6b:** *98186*, PISI/b. **8:** *98098*, THPL/b; *98102*, PISI/b. **18a:** *97241*, ABGR/b. **18b:** *97262*, ABGR/b (mixed with immature *Enerthenema* sp., probably *E. papillatum*). **19:** *97327b*, PSME/b. **20:** *98414, 98419, 98426, 98427, 98417*, ABGR/b (the last specimen mixed with immature *Licea* sp.). **22b:** *97219b*, QUGA/b; *97227*, QUGA/w; *97287a*, QUGA/w (mixed with *Comatricha ellae*); *97289b*, QUGA/w (mixed with immature *Comatricha* sp.). **24c:** *98037a*, QUGA/w (mixed with *Arcyria incarnata* and *Comatricha nigra*). **39b:** *98058*, PSME/w. – All specimens developed in moist chamber culture.

**∗*Arcyria versicolor*** Phill. **29:** *98507*, ABLA/b. **35:** *97041*, COSP/w. – According to Peck and Gilbert (1932) *A. versicolor* is rare at low elevations in NW Oregon but common at higher altitudes, especially in the high Cascades near the Three Sisters.

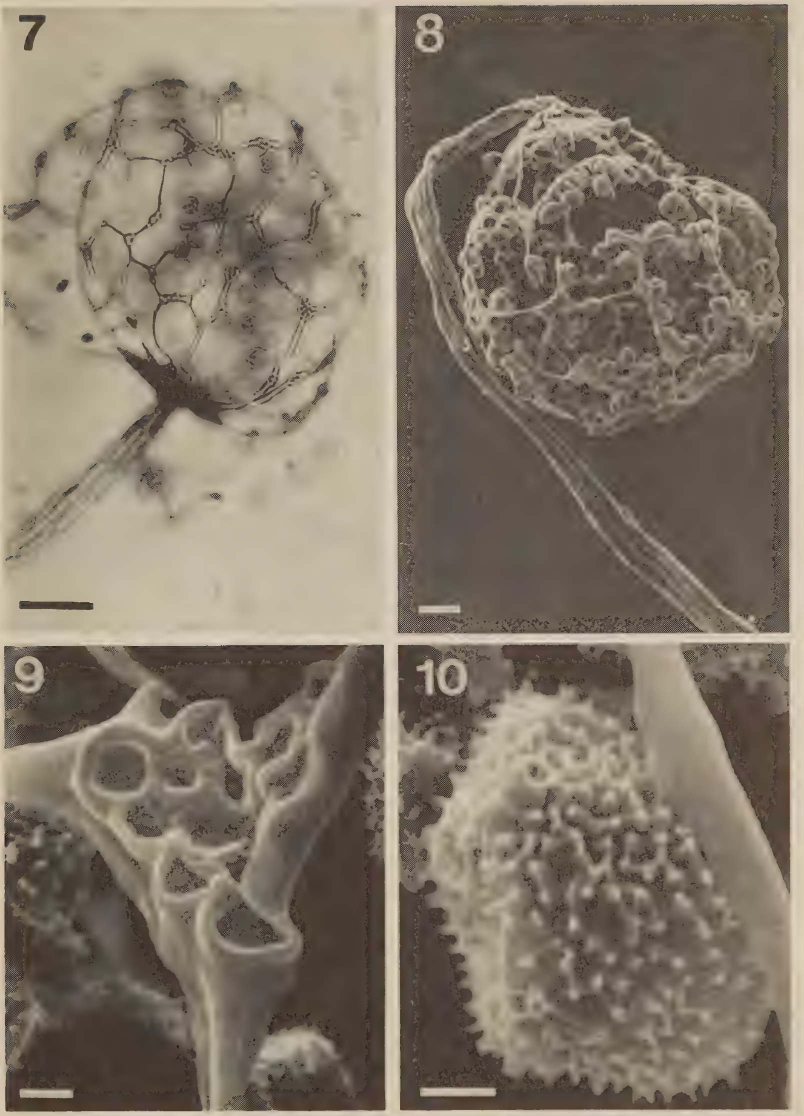
**∗*Metatrichia floriformis*** (Schwein.) Nann.-Bremek. **5a:** *98152*, PISI/w. **7b:** *98257*, PSME/w. **10b:** *98311A*, COSP/w. **11:** *98137Ab, 98137Cd, JRL137Eb, 98137Fc, 98137Gb*, COSP/w. **13:** *98286F*, COSP/w.

### **Family Trichiaceae** Rostaf.

**∗*Trichia botrytis*** (J. F. Gmek.) Pers. **2d:** *97140*, PISI/w. **3:** *98346*, PISI/w; *98353*, COSP/w. **6b:** *98178Bb*, THPL/b (mixed with *Lamproderma columbinum*). **4:** *97310*, PISI/w. **11:** *98137Da, 98137Ea*, COSP/w. **13:** *98286B*, ALRU/w. **18b:** *97246D*, COSP/w. **18c:** *97268*, COSP/b; *97269Bb*, COSP/b and w; *98281A*, ACMA/w. **22a:** *97189B*, QUGA/w. **24a:** *97124, 97125*, QUGA/w.

**∗*Trichia contorta*** (Ditmar) Rostaf. var. *contorta* **26:** *98622*, ABSP/w.

**∗*Trichia decipiens*** (Pers.) T. Macbr. **11:** *98137Cc*, COSP/w; the specimen represents the variety *olivacea* Meylan. **18a:** *97228a*, COSP/w (mixed with *Lamproderma columbinum*). **18b:** *97244*, COSP/w; *97251D* (var. *olivacea*), *97251Eb (*var. *olivacea*), ACMA/w (the later specimen mixed with *Physarum nutans*).



Figures 7–10. *Cribraria* sp. (*JRL97192D*). Figs 8–10 SEMs. Fig. 7. Head of sporangium in light microscope. Fig. 8. Nodding sporangium. Fig. 9. Detail of node in the peridial net. Fig. 10. One collapsed spore attached to the peridial net. Some of the spines of the spore wall unite into ridges. Scale bars: Fig. 7 = 25 µm, Fig. 8 = 10 µm, Figs 9–10 = 1 µm.

∗***Trichia favoginea*** (Batsch) Pers. emend M. L. Farr **2d:** *97141*, PISI/b. **11:** *98137Fa*, COSP/w. **18c:** *97264*, COSP/w; *97267*, COSP/bark of exposed roots.

∗***Trichia lutescens*** (Lister) Lister **18b:** *97254Aa*, ACMA/w (mixed with *Stemonitopsis typhina*). **18c:** *97272Ba*, detritus under epiphytic bryophytes on branches of large *Acer macrophyllum*; *97278A*, detritus under epiphytic bryophytes on basal trunk of young *Pseudotsuga menziesii*. – All our specimens are very scanty, the two last mentioned collections were made from moist chamber culture samples before wetting. Peck and Gilbert (1932) reported one specimen, which probably represented the first record of the species from North America, from a wet area in the Willamette Valley.

∗ ***Hemitrichia abietina*** (Wigand) G. Lister (syn. *Arcyria abietina* (Wigand) Nann.-Bremek.) **2a:** *97134*, trunk of living *Metasequoia glyptostroboides*. **22a:** *97182*, QUGA/w. – A rather rare species, found by Peck and Gilbert (1932) from only one locality in the Willamette Valley.

∗***Hemitrichia clavata*** (Pers.) Rostaf. **13:** *98286D*, ALRU/w. – Only one collection of this species, which one would expect to be common in the area studied. According to Peck and Gilbert (1932), it is abundant both in the Willamette Valley and in the Coast Range.

∗***Hemitrichia montana*** (Morgan) T. Macbr. OREGON. Umatilla Co., Blue Mtns., near La Grande. – The authors have studied two specimens collected by Maggie Rogers. According to Curtis (1970) this species is very common in Crater Lake National Park at elavations from 1830 to 2132 metres.

**Family Physaraceae** Rostaf.

∗***Fuligo intermedia*** T. Macbr. **10a:** *98292*, PSME/w. **10b:** *98311C*, fern litter. **34:** *98612*, ABSP/w. – Peck and Gilbert (1932) reported one specimen of *F. intermedia* from Oregon, collected on an old log in the foothills of the Cascades. Martin and Alexopoulos (1969) mention that, according to Kowalski, it is the most common species of *Fuligo* in northern California.

∗***Fuligo septica*** (L.) Wiggers **14:** *97158*, COSP/w. – Only one specimen of this cosmopolitan, common, and easily detectable species.

∗***Badhamia foliicola*** Lister **33:** *98D2*, ground litter, mc. – Peck and Gilbert (1932) reported only one collection of this species, which was found on small branches of oak in the Willamette Valley.

***Badhamia nitens*** Berk. **22b:** *97202A, 97202C, 97202D,* QUGA/w. – According to Peck and Gilbert (1932) this rare species is occasional during the winter rains in the Willamette Valley, usually growing on the bark of oak or alder logs. *Badhamia nitens* var. *reticulata* G. Lister has spores in loose clusters, wide elliptical or nearly globose, evenly covered with warts. Lizarraga, Moreno & Illana (1997) have proposed the variety *aurantiaca* Lizarraga, G. Moreno & Illana, which is characterized by an orange, one-layered, and roughened peridium. Our material from Oregon represents a typical *B. nitens.*

***Badhamia utricularis*** (Bull.) Berk. **20**: *98411Ab*, mosses, litter and thin twigs (mixed with *Physarum leucophaeum*).

***Physarum leucophaeum*** Fr. **18b**: *97255,* ACMA/w. **20**: *98411Ab*, mosses, litter and thin twigs, (mixed with *Badhamia utricularis*). **22a**: *97185, 97186, 97187A,* QUGA/w.

***Physarum nutans*** Pers. **18b:** *97251Ea,* ACMA/w (mixed with *Trichia decipiens* var. *olivacea*). **22a:** *97196,* QUGA/w.

***Physarum pusillum*** (Berk. & M. A. Curtis) G. Lister **19:** *97328,* ACMA/b, mc. – Peck and Gilbert (1932) reported *Physarum nodulosum* Cke & Balf as rare in Willamette Valley. In Martin and Alexopoulos (1969), this species is not mentioned, but *P. nodulosum* (Massee) Macbr. is listed as a synonym of *P. pusillum.*

***Physarum viride*** (Bull.) Pers. **12a:** *97165Cb,* PSME/b (mixed with *Calomyxa metallica*). **18b:** *97256,* ACMA/w; *97285,* COSP/w. **18c:** *97265Ba,* COSP/bark of exposed roots (mixed with *Licea pygmaea*). **23:** *97166Ba,* PSME/b (mixed with *Stemonitis* sp.).

***Leocarpus fragilis*** (Dicks.) Rostaf. **34:** *98605,* ABSP/w.

### Family Didymiaceae Rostaf.

***Didymium bahiense*** Gottsb. **19:** *97341,* TABE/b, mc.

***Didymium difforme*** (Pers.) S. F. Gray **13**: *98255D5,* grassy ground litter. 19: *98371A, 98371D,* slug dung on basal trunk of *Alnus rubra*; *98371Fb,* slug dung on basal trunk of *Abies grandis* (mixed with *Didymium iridis*). – All specimens developed in moist chamber culture. Nannenga-Bremekamp (1966) recognized *D. difforme* var. *comatum* Lister as a distinct species, *D. comatum* (Lister) Nann.-Bremek., based on slightly smaller, paler spores with small, usually united warts, and abundant, elastic capillitium composed of thin threads. According to Martin and Alexopoulos (1969), these two species seem to intergrade completely. This is also the case in our material from Oregon. Even in a single specimen (especially *98371D*), some sporangia had an abundant capillitium of thin threads and a dark brown spore mass, while in others the capillitium was scanty with thick threads and the spore mass was black. In both cases, the spores had a clear paler area, typical of *D. difforme*. Macroscopically, all fructifications were similar. Combinations with a dark brown spore mass and a scanty capillitium with thick threads, and a black spore mass with a scanty capillitium with thin threads also were included in the Oregon material. However, elsewhere (e.g. Finland) these two species seem to be clearly separable (Härkönen, pers. comm., specimens deposited in H).

***Didymium iridis*** (Ditmar) Fr. **18c**: *97271Ba*, detritus under epiphytic bryophytes on trunk of living *Pseudotsuga menziesii* (mixed with *Didymium squamulosum*). **19**: *98371E, 98371Fa* (mixed with *Didymium difforme*), slug dung on basal trunk of *Abies grandis*; *98371Ga*, slug dung in basal cavity of *Acer macrophyllum* (mixed with *Didymium squamulosum*). **24c**: *98045*, FRLA/b. – All specimens developed in moist chamber culture.

****Didymium minus*** (Lister) Morgan **20:** *98411C*, bark. – Peck and Gilbert (1932) considered this cosmopolitan species as rare in NW Oregon; they reported it on moss during the winter rains in the Willamette Valley.

****Didymium squamulosum*** (Alb. & Schwein.) Fr. **18c:** *97267Aa, 97268Ab, 97269D*, ground litter (mixed with *Arcyria cinerea*); *97268B, 97268D, 97269A, 97269C, 97269E*, ground litter; *97271Bb, 97273B*, detritus under epiphytic bryophytes on trunk of *Pseudotsuga menziesii* (the first specimen mixed with *Didymium iridis*); *97272Bb, 97274B*, detritus under epiphytic bryophytes on lower branches of large *Acer macrophyllum*; *97280*, ACMA/w (mixed with *Cribraria violacea*). **19:** *97340a*, TABE/w (mixed with *Arcyria cinerea* and *Licea biforis*); *98366*, FRLA/b; *98371B*, slug dung on basal trunk of *Alnus rubra*; *98371Gb*, slug dung in basal cavity of *A. macrophyllum* (mixed with *Didymium iridis*). **22a:** *97291D*, leafy ground litter; *97295B*, detritus under epiphytic bryophytes on branches of *Quercus garrayana*; *97299A, 97300A, 97301A, 97302A, 97303A*, detritus under epiphytic bryophytes on trunk of *A. macrophyllum*; *97300B, 97301B, 97302B, 97303B*, detritus under epiphytic bryophytes on branches of *A. macrophyllum*. **22b:** *97293A, 97295A, 97297A*, detritus under epiphytic bryophytes on basal trunk of *Q. garrayana*; *97286A, 97286Ca, 97286D, 97286E, 97286B*, leafy ground litter (the last specimen mixed with *Arcyria cinerea*). **23:** *97210*, ACMA/b (mixed with immature *Physarum* sp.). – All specimens developed in moist chamber culture.

****Didymium*** cf. ***trachysporum*** G. Lister **33:** *98D8*, ground litter, mc. – The specimen is very scanty. *Didymium trachysporum* has white, pale grey or cream-coloured sporangia, a reduced columella, and a fairly scanty capillitium (Martin & Alexopoulos, 1969; Nannenga-Bremekamp, 1991; Neubert, Nowotny & Baumann 1995). The two sporangia in the Oregon specimen are pale ochraceous brown, have a dark brown and shiny columella and a fairly abundant capillitium. The capillitial threads, however, strongly resemble those of *D. trachysporum*, having some dark, nodular thickenings and expansions enclosing calcareous crystals. The spores (9–11 µm diam.) also fit *D. trachysporum*. *Didymium quitense* (Pat.)

Torrend is similar in appearance, but the spores are more densely warted and larger, (12–)14–15 µm in diam. (Nannenga-Bremekamp 1991).

Peck and Gilbert (1932) reported that this species was found several times in Mr. Gilbert's garden on the surface of daffodil bulbs about four inches underground. They suspected that it had been transported on bulbs imported from Holland.

*__*Lepidoderma tigrinum*__ (Schrad.) Rostaf. **18b:** *97252A, 97252B*, PSME/b. **18c:** *97281B*, ACMA/w. **19:** *97344*, PSME/w. – According to Peck and Gilbert (1932), this species is rare on well rotten wood during winter rains in the Willamette Valley.

**Family Elaeomyxaceae** Hagelst. ex M. L. Farr & H. W. Keller
***Elaeomyxa cerifera*** (G. Lister) Hagelst. (Figs 11-15). **2d:** *97150*, PISI/w. – We have found no earlier records of this rare species from temperate North America. However, it has recently been reported from Mexico (Rodriguez Palma, 1998). Sporangia in small groups, stalked to nearly sessile, globose to subglobose, iridescent; total height 1.2–1.5 mm, 0.8–1.5 mm in diameter. Stalk up to half of the total height, thick, black, opaque in transmitted light; apex widened as a collar-like structure, in some sporangia yellow or red-brown, filled with wax. Hypothallus fairly large, membranous, brown. Peridium thin, membranous, iridescent in blue, red and green; dehiscing into large flakes but persisting at the base. Columella absent. Capillitium radiating from the apex of the stalk, dense, rigid, dark; threads roughened, thick (2–3 µm in diam.), paler at the base, tapered and colourless at the apex, dichotomously branching and with some interconnections in the apical parts. Spores blackish brown in mass, pale greyish brown in transmitted light, globose, subglobose to broadly elliptical, covered with dispersed spines, 10–11–11.5 µm in diameter.

**Other material studied**. Switzerland: The lectotype of *Diachea cerifera* G. Lister var. *sessilis* Meylan, Les Forets du Creux du Van, Canton Vaud, Oct. 1931, C. Meylan, (LAU).

**Family Stemonitaceae** Rostaf.
* ***Colloderma oculatum*** (Lippert) G. Lister **4:** *97309E*, COSP/w, mc. – A scanty specimen existing only as a permanent slide. Peck & Gilbert (1932) did not report this species from Oregon, but according to Martin & Alexopoulos (1969), it has been collected from the state.

*__*Symphytocarpus flaccidus*__ (Lister) Ing & Nann.-Bremek. **18a:** *97234*, PSME/w. **28a:** *97028B*, PICO/w. **28b:** *97034, 97035*, PSME/w; *97037*, PSME/b **41a:** *97065, 97066*, PSME/b. – All specimens developed in moist chamber culture.

∗***Stemonitis axifera*** (Bull.) T. Macbr. **19:** *97329*, ALRU/w, mc. **22a:** *97188*, QUGA/w. **25:** *97005*, ABPR/w.

∗***Stemonitis fusca*** Roth **18a:** *97238*, ABGR/b, mc; **20:** *98411B*, mosses, litter and thin twigs. **22a:** *97187B*, QUGA/w.

∗***Stemonitis*** cf. ***pallida*** Wingate **13:** *98400*, bark of thin twigs, mc. – *Stemonitis pallida* has violet-brown to dark brown or dusky drab sporangia with sporangia with a stalk about one fourth to one half of the total height (Martin & Alexopoulos, 1969; Farr, 1976; Nannenga-Bremekamp, 1991). According to Nannenga-Bremekamp (1991), it differs from *Stemonitis herbatica* Peck in bearing small spines in a more delicate capillitial surface net. *Stemonitis herbatica* typically grows on plant remains and living plants, but it also occasionally occurs on wood. Farr (1976) described the surface net of *S. pallida* to be smooth and more delicate than that of *Stemonitis flavogenita* Jahn, which is close to both *S. pallida* and *S. herbatica*. The capillitial internal net of *S. flavogenita* has many membranous expansions, the surface net usually has many spine-like free ends, the columella often ends as a membranous expansion, and the plasmodium is yellow. Fructifications in the Oregon specimen are bright brown to ferruginous in colour, the stalks are very short, the inner capillitial net has expansions, and the very delicate, small-meshed capillitial surface net is smooth.

***Macbrideola martinii*** (Alexop. & Beneke) Alexop. **24c:** *98041a*, QUGA/b, mc (mixed with *Licea* sp. 1).

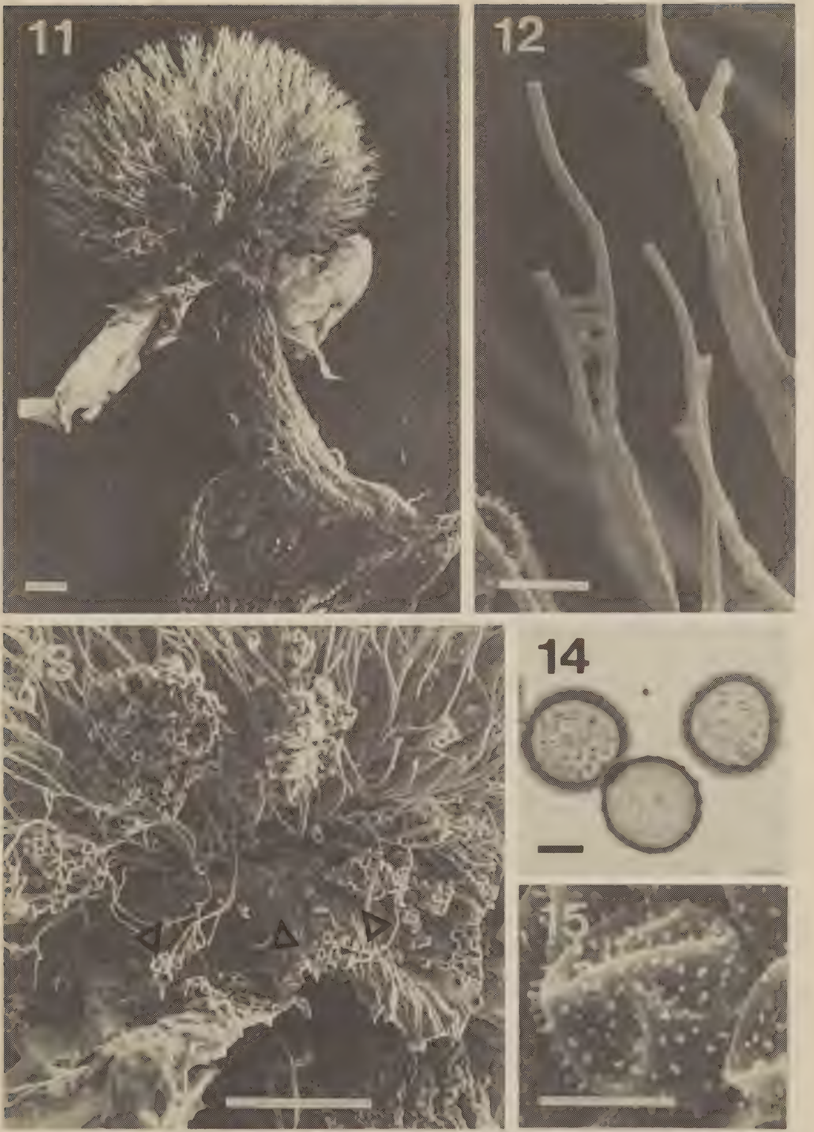
∗***Stemonitopsis typhina*** (Wiggers) Nann.-Bremek. **18b:** *97254Ab, 97254B*, COSP/w (the first specimen mixed with *Trichia lutescens*).

***Comatricha alta*** Preuss **12c:** *982234*, PSME/w. **17:** *98004a*, ALRU/w.

∗***Comatricha elegans*** (Racib.) G. Lister **5a:** *98140*, PISI/b, mc. **13:** *98403*, PSME/w, mc. **19:** *97327a*, PSME/b; *97335*, PSME/b, mc. **26:** *98668*, ABSP/w, mc. **30:** *98562b*, PIPO/w. **39b:** *98055b*, CADE/w, mc (mixed with *Comatricha ellae* and *C. nigra*).

***Comatricha ellae*** Härk. **17:** *98023a, 98026*, ABGR/b, mc. **22b:** *97287b*, QUGA/w, mc (mixed with *Arcyria pomiformis*). **25:** *97014a*, ABPR/w. **28b:** *97031A*, PSME/w. **32:** *98327*, PIPO/w, mc (mixed with immature *Enerthenema* sp.). **35:** *97040*, COSP/w, mc. **37a:** *98590, 98591*, PIAL/w, mc. **38b:** *98610*, COSP/w, mc. **39b:** *98055a*, CADE/w, mc (mixed with *Comatricha nigra* and *C. elegans*). **39d:** *98081b*, PIJE/w, mc (mixed with *Enerthenema papillatum*). **40b:** *97063*, PSME/b.

∗***Comatricha laxa*** Rostaf. **12a:** *97164*, THPL/b. **17:** *98015*, COSP/w. **22b:** *97203c*, QUGA/b and w (mixed with *Licea minima* and *Stemonitis* sp.). **28b:** *97032a*, PSME/w (mixed



Figures 11–15. *Elaeomyxa cerifera* (*JRL97150*). Figs 11–13, 15 SEMs. Fig. 11. Sporangium with dehiscent peridium persisting at base of the spore-case. Fig. 12. Tips of capillitial threads. Fig. 13. Widened, collar-like apex of the stalk, from which the capillitium arises (arrows). Fig. 14. Spores in light microscope. Fig. 15. A collapsed, spiny spore. Scale bars: Figs. 11, 13 = 100 μm, Figs 12, 14, 15 = 5 μm.

with *Enerthenema papillatum*). **32:** *98328b*, PIPO/w (mixed with *Enerthenema papillatum*). **41b:** *97073*, QUGA/w.

∗***Comatricha longa*** Peck **6b:** *98183*, PISI/b, mc. – Peck and Gilbert (1932) reported this species as rare in NW Oregon. Their specimen was collected on willow logs near the Willamette River.

∗***Comatricha nigra*** (Pers.) Schroet. **1:** *98443*, PISI/w, mc. **2b:** *97132A*, PISI/w. **2c:** *97135B, 97136A, 97136B*, PISI/w. **4:** *97308*, TSHE/b. **5a:** *98144*, PISI/b, mc; *98151*, PSME/b, mc. **6a:** *98162b*, PISI/b, mc. **6b:** *98178*, THPL/b, mc. **10a:** *98288*, THPL/b; *98299*, TSHE/b. **11:** *98117, 98120*, PSME/w. **13:** *98275*, PSME/w; *98286E*, SARA/w. **16:** *98219*, THPL/w. **17:** *98016*, PSME/b, mc. **18a:** *97230*, PSME/b; *97234B, 97237*, PSME/w. **18b:** *97258*, ACMA/w. **19:** *97331*, PSME/b, mc; *98369b*, ABGR/b, mc (mixed with immature *Licea* sp.). **22a:** *97189A*, QUGA/w. **22b:** *97170, 97174*, QUGA/w. **23:** *97208*, ACMA/w, mc. **24b:** *97117*, PSME/b. **24c:** *98036*, COSP/w, mc; *98037c*, QUGA/w, mc (mixed with *Arcyria incarnata* and *A. pomiformis*); *98040a*, COSP/w. **25:** *97022a*, ABPR/w. **26:** *98673*, TSHE/b, mc. **27:** *97043*, PSME/b, mc. **28b:** *97033a*, PSME/w (mixed with *Enerthenema papillatum*). **32:** *98312*, COSP/w, mc; *98313*, PIPO/w; *98320A*, COSP/b, mc; *98324*, ABSP/w, mc; *98329*, PIPO/w, mc; *98331*, PSME/b, mc. **34:** *98643*, CHNO/w, mc; *98655*, TSME/b, mc. **38a:** *98603*, TSME/w, mc. **38c:** *98631*, PIMO/b, mc. **39b:** *98055c, 98069*, CADE/w, mc (the first specimen mixed with *Comatricha elegans* and *C. ellae*); *98057a*, PIJE/w, mc (mixed with *Echinostelium minutum*). **40a:** *97055, 97056*, CADE/w, mc. **41a:** *97067b*, PSME/b (mixed with *Enerthenema papillatum*). **41b:** *97072*, ARME/w. **41c:** *97074*, PSME/w.

***Comatricha nigricapillatum*** (Nann.-Bremek. & Bozonnet) A. Castillo, G. Moreno & Illana 35: *97039, 97042*, COSP/w. – Nannenga-Bremekamp and Bozonnet (in Nannenga-Bremekamp 1989) described this species as *Lamproderma nigricapillatum* Nann.-Bremek. & Bozonnet. According to Castillo, Moreno, Illana & Lago (1997), this species belongs in *Comatricha* because of the early evanescent peridium (leaving only a collar and some persistent silvery flakes), and the capillitium arising not only from the apex of the columella but also from the upper half. Castillo *et al.* (1997) consider *Collaria chionophila* Lado (Lado, 1992) as a synonym.

**Other material studied**. France: Col de la Madaleine, 1 June 1993, herb. Marianne Meyer 13 441 (dupl. in H); Les Arcs, 25 May 1997, herb. Marianne Meyer 18 289 (dupl. in H).

∗ ***Comatricha pulchella*** (Bab.) Rostaf. OREGON. Lane Co.: **Eagles Rest:** Old-growth *Pseudotsuga menziesii* forest on ridge. 43°85′N, 122°75′W. Elev. 820 m. *EP12-3Aa* (mixed with *Licea* cf. *castanea*).

∗***Enerthenema papillatum*** (Pers.) Rostaf. **2b:** *97128A, 97133*, PISI/w. **8:** *98086a, 98086b*, COSP/w, mc. **12b:** *97176A*, FRLA/w. **18a:** *97234Ca*, PSME/w (mixed with *Comatricha* sp.). **18b:** *97257A, 97257B*, ACMA/w. **19:** *97346A, 97346Ab*, mc, *97346B*, COSP/w. **22a:** *97193*, COSP/w. **24b:** *97119*, detritus and soil in trunk cavity of *Pseudotsuga menziesii*. **28b:** *97032b, 97033b*, PSME/w (mixed with *Comatricha laxa* and *C. nigra*). **30:** *98566*, PIPO/w, mc. **32:** *98327, 98328a*, PIPO/w (the latter specimen mixed with *Comatricha laxa*). **39b:** *98059, 98064*, PIJE/w, mc. **39d:** *98081a*, PIJE/w, mc (mixed with *Comatricha ellae*). **41a:** *97070b*, PSME/w (mixed with immature *Comatricha* sp.); *97067a, 97069*, PSME/b (the first specimen mixed with *Comatricha nigra*). – Two specimens (*97346A* and *97346B*) are atypical in having a capillitium arising along the whole columella, as in *Enerthenema intermedium* Nann.-Bremek. (described from California). However, the sporangia of *E. intermedium* are deep black and spores are dark with a pale side (Nannenga-Bremekamp & Critchfield, 1988). In this respect *E. intermedium* resembles *E. melanospermum* T. Macbr. & G. W. Martin (described from the Cascade Mountains), but the latter species is larger and has a very distinct apical plate. The authors have seen the type of *E. melanospermum*.

***Paradiacheopsis fimbriata*** (G. Lister & Gran) Hertel **22b:** *97222a*, PSME/b; *97288*, PSME/b, mc. **39c:** *98076*, CHLA/w, mc. **39d:** *98083*, PSME/b and w, mc.

***Paradiacheopsis rigida*** (Brândză) Nann.-Bremek. **13:** *98403*, PSME/w.

***Paradiacheopsis solitaria*** (Nann.-Bremek.) Nann.-Bremek. **24a:** *97123*, PSME/b.

****Lamproderma columbinum*** (Pers.) Rostaf. **6b:** *98178A, 98178Ba*, THPL/b (the latter specimen mixed with *Trichia botrytis*). **8:** *98087*, COSP/w. **10b:** *98311D*, COSP/w. **11:** *98137Aa, 98137Ba*, COSP/w. **13:** *98255B4, 98286A*, COSP/w; *98286G*, PSME/w. **18a:** *97228b*, COSP/w (mixed with *Trichia decipiens*). **18b:** *97243, 97246Ba*, COSP/w.

### Species richness and productivity

There were clear differences in the diversity and abundance of myxomycetes among the different forest zones (Tables 1 & 2). Both species richness and the productivity of moist chamber cultures were highest in the forests and woodlands of the Willamette Valley. The greatest number of species was found in foothill forests, while the productivity of moist chamber cultures was highest in the woodlands of the valley bottom. The forests of the Klamath region were also quite productive. The total number of myxomycete species was low, but this may have mainly been a reflection of the limited number of field collections. Species accumulation values for Klamath forests (0.20 and 0.19 species/field sample in the Oregonian and Californian sites, respectively) were comparable to those recorded in the Willamette Valley (0.23 and 0.18 species/field sample in foothill and bottomland forests, respectively).

Species richness and productivity in moist low elevation coniferous forests were low, especially when considering the relatively high sampling intensity in these forest types. Species accumulation values in the *Picea sitchensis* and *Tsuga heterophylla* zones were identical (0.08 species/field sample) and also the productivity of moist chamber cultures was remarkably low in both zones, but especially so in the *Tsuga heterophylla* zone (Table 2).

High elevation coniferous forests on both sides of the Cascade Crest where similar with respect to species richness and productivity of moist chamber cultures. Species richness was low, with similar species accumulation values on both slopes (0.11 and 0.14 species/field sample W and E of the Crest, respectively). However, the productivity of moist chamber cultures was clearly higher west of the Crest. Subalpine coniferous forests were characterized by low species richness and species accumulation values (0.09 species/field sample). The productivity of moist chamber cultures in these high elevation forests was nearly as low as in the *Picea sitchensis* and *Tsuga heterophylla* zones (Table 2).

## DISCUSSION

Our study adds 29 myxomycete species to the lists given by Peck and Gilbert (1932), and by Curtis (1970) for Oregon. Since Peck´s and Cilbert´s paper comprises the results of collections made during some twenty years, their list naturally includes many rare species of the Liceales, Trichiales, and Stemonitales that were not found by us. However, some presumably common, cosmopolitan species also are missing from our list. Prominent examples include *Cribraria argillacea*, *Lamproderma violaceum*, *Perichaena corticalis*, *Stemonitis flavonenita*, and *Trichia varia*. *Ceratiomyxa fructiculosa* was surprisingly rare, and the abundance and diversity of *Echinostelium* also were both quite low. Wide temporal fluctuations in the occurrences of myxomycete species may explain some of these unexpected absences. The diversity of the genus *Echinostelium* has been found to be high in Mediterranean regions (Whitney, 1980; Whitney, 1982; Härkönen and Uotila, 1983; Härkönen, 1987; Lado, 1994; Pando, 1997) but low both in boreal regions and in the tropics (Farr 1976; Härkönen, 1977; Härkönen, 1978; Batista, da Silva & Minter, 1995; Ukkola, 1998c).

The total productivity of our moist chamber cultures (25.5%) was low but varied considerably between different vegetation zones. When all stages of myxomycetes were included, the productivity increased to 38.4%. When collecting, the second author focused on habitats and substrates (i.e., different types of bark and wood) favourable for members of the Caliciales. Many calicioid fungi prefer the basal trunks of old, slow-growing trees in mature or old-growth forests. Patches of exposed wood on scarred trees provide another important habitat type for lignum-inhabiting species, and dry snags in open situations also are favoured by several species. Conversely, some calicioid fungi require microhabitats (e.g., deep trunk crevices or root overhangs of uprooted trees) with a constantly high atmospheric humidity and low light intensity. However, even these species tend to avoid substrates with high water retention capacities, such as soft rotten wood with epixylic liverworts and other bryophytes. The strong focus on calicialean microhabitats undoubtedly explains why our list includes relatively few species of *Didymium* and *Physarum*, and no species of *Craterium*. Species in these genera are mostly found on litter and plant remains. While several types of litter and detritus were collected for moist chamber cultures, the total number of cultures may have been too limited or the incubation time too short to bring out these genera. According to Peck and Gilbert (1932), *Physarum bitectum*, *P. cinereum*, *P. leucophaeum*, *P. mortoni*, *P. notabile*, *P. nutans*, and *P. viride* should be common in NW Oregon, but only three of these species were found by us.

Some species of *Diderma* are frequently found on bark or wood, including *D. radiatum* which has been found on many types of wood and leaf litter. However, our material does not include any specimens of *Diderma*, and it is actually quite surprising that no members of this genus developed from the 780 moist chamber cultures prepared in our study. One can again refer to the possibly insufficient incubation time, although previous moist chamber cultures of Tanzanian material did produce *Diderma* species in four weeks (Ukkola, 1998a; Ukkola, 1998b; Ukkola, 1998c). According to Ing (1994), some species of myxomycetes may take several months to develop plasmodia and produce spores. In our material, the first 159 moist chamber cultures were re-wetted and incubated for an additional four weeks following two weeks of dryness. In these cultures, plasmodia and mature fructifications were usually produced during the first incubation period and the number of myxomycete species did not increase significantly during the second incubation period. However, in some cases plasmodia did not sporulate even if the cultures were incubated for several extra weeks.

Among our collecting sites, myxomycete diversity and productivity increased with increasing heterogeneity in forest structure, especially with increasing availability of angiospermous bark and wood as potential substrates for myxomycetes. For example, the productivity of moist chamber cultures from maritime low elevation conifer forests (*Picea sitchensis* and *Tsuga heterophylla* zones) was remarkably low. Substrate samples from these forests included many samples of wood and bark of *Picea sitchensis*, *Pseudotsuga menziesii*, and *Tsuga heterophylla*, which obviously were not favourable substrates for myxomycetes. Previous studies of corticolous and lignicolous myxomycetes have usually found low correlations between myxomycetes and specific tree species, but conifers have often been less productive than broadleaf trees (Härkönen, 1977; Härkönen & Uotila, 1983; Stephenson, 1989). For example, in the British Isles, bark of *Acer*, *Fraxinus*, and *Quercus* were quite productive while those of conifers were generally poor (Ing, 1994). A similar situation was seen in Oregon, where *Quercus garrayana*, *Acer macrophyllum*, *Fraxinus latifolia*, and *Alnus rubra* were quite productive, but *Pseudotsuga menziesii*, *Picea sitchensis*, *Tsuga heterophylla*, and *T. mertensiana* were generally unproductive. *Abies grandis, A. procera*, and *Pinus ponderosa* were somewhat more productive than the other conifers, but not as productive as angiosperms. Many epiphytic lichens differ in frequency between conifers and hardwoods (e.g., McCune & Geiser, 1997). This variation is related to various attributes in substrate quality, including differences in bark chemistry between different tree species. Similar phenomena also are likely to be occur in myxomycetes. In our material, some types of conifer substrates were clearly very unfavourable for myxomycetes. For example, resin soaked wood and bark, which was the preferred substrate of several calicioid fungi, did not yield any myxomycetes in culture. It is also possible that toxic lichen substances, such as vulpinic acid, produced by many calicioid fungi and other epiphytic lichens may have acted to lower myxomycete productivity in some moist chamber cultures. Vulpinic acid-containing epiphytes are common in high elevation forests of the Pacific Northwest and many moist chamber cultures from these locations included fragments of their thalli.

This study confirms the results of some earlier studies (see e.g. Stephenson, 1989; Härkönen & Uotila, 1883; Ukkola *et al.*, 1996; Ukkola 1998a, b): in general the members of the order Physarales grow on less acid substrata than the members of the order Stemonitales. Many members of the orders Liceales and Trichiales have a fairly wide pH amplitude, although many species of the latter order prefer less acid substrata.

Differences in the duration and frequency of wetting and drying cycles may have been involved in creating some floristic differences among low elevation forests. Myxomycetes displayed highest levels of species richness and productivity in the mixed forests and deciduous woodlands of the Willamette Valley. These forests are located at the low end of the elevational gradient, but they are not as wet as neighbouring coniferous forests, mainly because of their location in the rain shadow of the Coast Range. Moreover, the forests of the Klamath region, which also displayed relatively high levels of myxomycete productivity, tend to be drier than coastal coniferous forests. This is partly because of the rain shadow of the Siskiyou Range, but also is due to open canopies on serpentine soils. It is possible that the moist chamber technique more accurately mimics the natural wetting and drying cycles of these forests than those of hypermaritime forests. In any case, myxomycetes in coastal forests must be adapted to higher overall moisture and less frequent wetting and drying than myxomycetes in the Willamette Valley or Klamath Region, respectively.

One clear trend in our material was that of decreasing myxomycete diversity and productivity with increasing elevation in montane forests. This finding agrees well with previous observations of the vertical zonation of myxomycetes and their association with montane vegetation types (Ing, 1994; Ukkola, Härkönen & Saarimäki, 1996; Ukkola, 1998c; Stephenson, Landolt & Moore, 1999). High elevation forests have relatively harsh growing conditions; especially in subalpine forests, low temperatures may play a significant role as a limiting factor for many myxomycete species.

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# NOTES ON HYPHOMYCETES. LXXVIII. ASPERISPORIUM SEQUOIAE, THE CAUSAL ORGANISM OF CONIFER NEEDLE BLIGHT, RECLASSIFIED IN CERCOSPORIDIUM, WITH COMMENTS ON THE STATUS OF THE GENUS

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## ABSTRACT

The plant-pathogenic anamorph currently known as *Asperisporium sequoiae* (Ell. & Everh.) Sutton & Hodges, which was originally classified in *Cercospora* Fries and occurs commonly as the causal organism of needle blight of Leyland Cypress [*Cupressocyparis leylandii* (Dallim & A.B. Jackson) Dallim] in Alabama, is considered to be more satisfactorily classified in *Cercosporidium* Earle and is, accordingly, transferred to that genus. It is also redescribed and illustrated. The distinctiveness and validity of the *Cercosporidium* generic concept is reevaluated and its status as an individual entity is upheld. The morphological discontinuities between it and *Passalora* Fries are considered to be sufficiently adequate to warrant separate recognition. Its circumscription is based upon a number of readily recognizable characteristics which make it easily distinguishable from related genera.

**KEYWORDS:** *Cercospora*-complex, *Passalora*, generic concepts.

## INTRODUCTION

Several fresh collections of *Asperisporium sequoiae* made in recent years on Leyland Cypress (the intergeneric hybrid between *Chamaecyparis nootkatensis* (D. Don) Spach. and *Cupressus macrocarpa* Hartw.) in Alabama has afforded an opportunity to study the fungus, as a result of which its present generic classification has been determined to be unsatisfactory. This anamorph causes a severe needle blight disease on a number of coniferous hosts in the southeastern United States, including *Cryptomeria japonica* D. Don, and species of *Cupressus* L., *Juniperus* L., and *Thuja* L. (Hodges, 1961; Sutton and Hodges, 1990). On Leyland Cypress the symptoms of infection are quite pronounced with progressive browning of infected needles occurring. Where plants are severely affected whole branches may become discolored, with resultant dieback. In Japan, *A. sequoiae* is especially damaging on *Cryptomeria japonica* (Ito *et al.*, 1967) and it is likewise quite severe on this host and its *elegans* (Henk. & Hochst.) Mast. variety in Brazil (Hodges and May, 1972). It is also known to occcur in several other countries (Sutton and Hodges, loc. cit.).

In considering an appropriate genus in which to classify *A. sequoiae*, having determined it not to belong in *Cercospora* Fres. on account of possession of mid-brown, verruculose conidia, Sutton and Hodges (loc. cit.) reviewed the characteristics of several possible alternative, segregate genera. These included *Asperisporium* Maubl., *Exosporium* Link, *Helminthosporium* Link, *Heterosporium* Klotzsch., *Pseudocercospora* Speg., and *Pseudospiropes* M.B. Ellis. Chupp (1954) had earlier entertained the notion of classifying it in *Heterosporium* because of the ornate nature of its conidia. It clearly, however, cannot be accommodated in that genus whose species are now, in any case, classified in *Cladosporium* Link (David, 1997) although some authors (von Arx, 1983; McKemy and Morgan-Jones, 1990) have argued in favor of maintaining the taxon as a separate entity. *Exosporium* was previously suggested by Hodges (1962) but neither it, nor *Helminthosporium*, are even remotely similar to *A. sequoiae*.

In their discussion of likely generic candidates Sutton and Hodges (loc. cit.) viewed *Pseudocercospora* as a “suitable” possibility but correctly rejected the genus because of the fact that *A. sequoiae* possesses thickened conidiophore scars. *Pseudospiropes* was similarly eliminated from consideration because the conidia of *A. sequoiae* are euseptate and quite unlike the distoseptate conidia of the type species of that genus, *P. nodosus* (Wallr.) M.B. Ellis. In adopting *Asperisporium* as the nomenclatural entity in which to accommodate the anamorph under consideration herein, Sutton and Hodges (loc. cit.) stated that it conformed, to quote, “exceedingly well” with the criteria by which that genus is characterized except for the fact that it does not form the sporodochial conidiomata which are typical. Indicating, however, that perhaps the authors were not entirely satisfied with this chosen classification it was stated, again to quote, that “For the moment *Asperisporium* is a far preferable generic placement for *C. sequoiae* than *Cercospora*, *Pseudocercospora*, or *Pseudospiropes*.”

We agree with this sentiment but nevertheless question the suitability of this taxonomic placement. *Cercospora sequoiae* differs from the type species of *Asperisporium*, *A. caricae* (Speg.) Maubl., in several additional, significant respects. The conidiophores are arranged in a loose fascicle rather than being tightly packed and their morphology is quite different as a result of substantial sympodial growth. Moreover, the shape of the conidia is quite dissimilar, being cylindrical to very narrowly obclavate rather than ellipsoidal or obovoid. Although the conidia of one or two taxa included in *Asperisporium*, such as *A. cassiae* (H. Syd.) Deighton and *A. moringae* (Thirum. & Govindu) Deighton, may be somewhat obclavate their length/width ratio is very different from those of *A. sequoiae*.

It is somewhat surprising that the genus *Cercosporidium* Earle was not considered, or even mentioned, by Sutton and Hodges (loc. cit). Apart from the fact that the conidia are not smooth, and are longer in relation to their width, *A. sequoiae* is very similar to *C. chaetomium* (Cooke) Deighton, the type species of *Cercosporidium*. Its stroma and particularly the morphology of its conidiophores are highly typical of those characteristic of this genus. The incurved conidiophore growth pattern and the presence of a thickened band along one side, two features peculiar to *Cercosporidium*, are well exemplified in *A. sequoiae*. A number of species classified in *Cercosporidium*, such as *C. punctiforme* (Wint.) Deighton, also have ornamented conidia. Moreover, a number of taxa classified in *Cercosporidium* have conidia of similar shape and length/width ratio to those of *A. sequoiae*. All things being considered, there can be little doubt that the anamorph originally named *Cercospora sequoiae* belongs in *Cercosporidium*. It is, in fact, highly typical of this genus and is, accordingly, transferred to it herein.

Some argument and disagreement currently exists in the literature with regard to the independent status of *Cercosporidium*. Several authors, such as von Arx (1983), Castañeda and Braun (1989), and Braun (1995), treat the genus as synonymous with *Passalora* Fr., maintaining presumably that there is no clear-cut morphological discontinuity between species classified in the respective genera, whereas others, such as Pons and Sutton (1996), adopt an opposing position. Although there may be something of a continuum between the two, the present authors, however, disagree with the contention that amalgamation is desirable and believe that *Cercosporidium* remains sufficiently well-defined to be perpetuated as a separate entity. A discussion relating to this is offered below and a case made for continuing to recognize the genus as distinct.

## TAXONOMIC PART

*Cercosporidium sequoiae* (Ell. & Everh.) Baker & Partridge, comb.nov. (Figure 1)

≡ *Cercospora sequoiae* Ell. &. Everh., *J. Mycol.* 3: 13, 1887.

≡ *Asperisporium sequoiae* (Ell. & Everh.) Sutton & Hodges, *Mycologia* 82: 314, 1990.

= *Cercospora cryptomeriae* Shirai *apud* Kitakima, *Bot. Mag.*, Tokyo 30: 413, 1916 [*fide* Ito *et al.*, 1967].

= *Cercospora thujina* Plakidas, *Phytopathol.* 35: 181, 1945.

Needle lesions brown, irregular, often becoming extensive, confluent and necrotic. Mycelium immersed primarily in the epidermis and subepidermal tissues, intracellular, composed of pale to mid brown, septate, branched, smooth, 3-5µm wide hyphae. Caespituli punctiform, usually gregarious and distributed predominantly on the margin of the symptomatic lesions, brown to dark brown. Stromata epidermal or subepidermal, pseudoparenchymatous, composed of mostly isodiametric cells, well-developed, dense, mostly discrete, globose to subglobose in shape, mid brown to brown, up to 60µm wide, commonly rupturing the stomata. Conidiophores arising from the outermost, upper, exposed cells of the stromatic tissue, macronematous, mononematous, fasciculate, commonly occurring in large, dense fascicles of twenty or more, simple, flexuous to somewhat tortuous, often incurved toward their apices and bearing an associated thickened band along one side of the periclinal wall, smooth or rarely verruculose, cylindrical, often becoming geniculate distally following sympodial growth associated with conidiation, 3-5 euseptate, brown to pale brown apically, 60-90 × 3-5µm. Conidiogenous cells integrated, terminal, polyblastic, sympodial, with occasional regenerative percurrent proliferations, cicatrized, pale olivaceous brown, bearing thickened scars. Conidia holoblastic, acrogenous, solitary, dry, subcylindric to narrowly obclavate, straight or slightly curved, attenuating gradually, obtuse at the apex, densely verruculose, pale olivaceous brown, 2-5 euseptate, tapering abruptly and bearing a thickened hilum at the subtruncate base, 36-63 × 4-6µm.

On living leaves or young twigs of × *Cupressocyparis, Cupressus*, *Juniperus*, *Thuja* [Cupressaceae], *Cryptomeria, Sequoia*, *Sequoiadendron*, and *Taxodium* [Taxodiaceae], species; cosmopolitan.

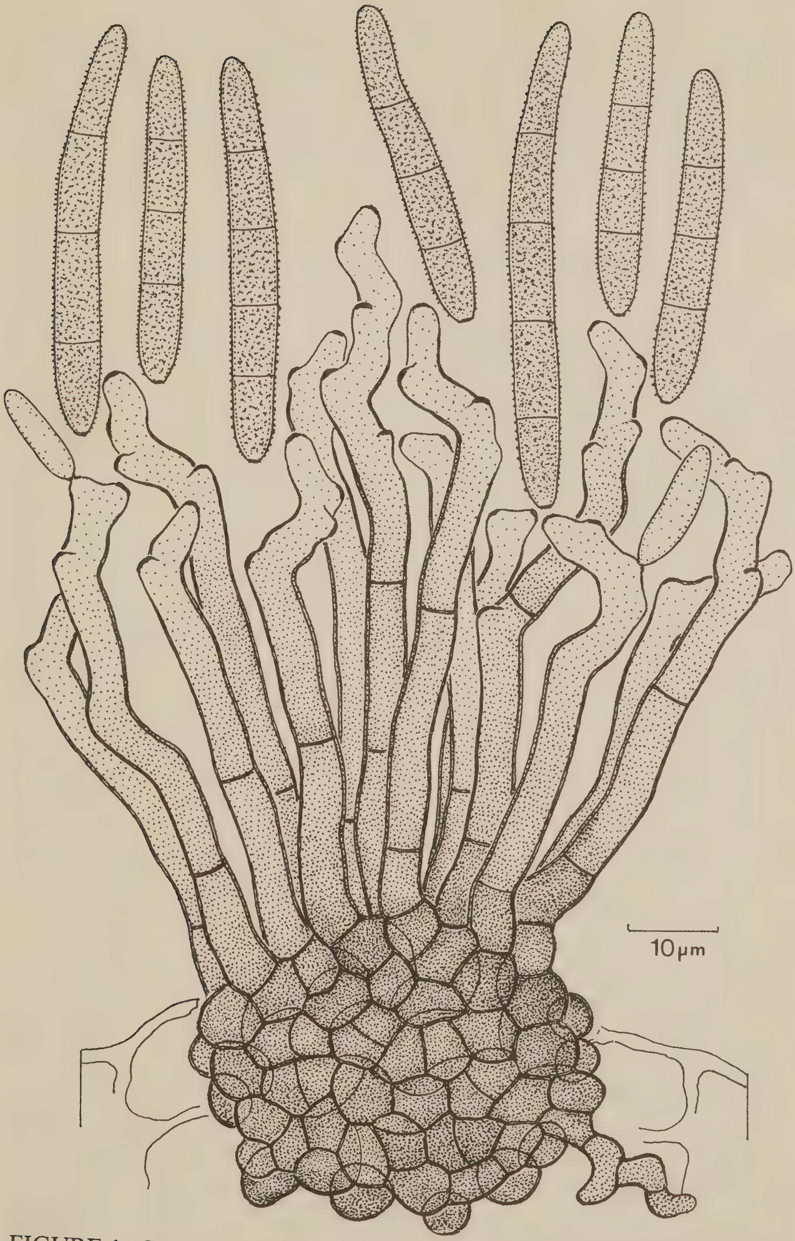

FIGURE 1. *Cercosporidium sequoiae.* Stroma, conidiophores and conidia.

Collections examined: on × *Cupressocyparis leylandii*, Bay Minette, Baldwin County, Alabama, U.S.A., 6 October 1994, J. Olive, AUA; on × *C. leylandii*, Mobile, Mobile County, Alabama, 9 May 1995, J.M. Mullen, AUA; on × *C. leylandii*, Montgomery, Montgomery County, Alabama, 26 February 1999, W.A. Baker, AUA; × *C. leylandii*, Mobile, Mobile County, Alabama, 2 November 1999, J.M. Mullen, AUA.

## COMMENTS ON CERCOSPORIDIUM

The generic name *Cercosporidium* has had a somewhat chequered history characterized by indecision on the part of taxonomic mycologists as to its distinctiveness and, in particular, as to whether or not it and *Passalora* should be recognized as separate entities. Such a situation exists even to this day. In fact, from early on in its very existence the status of *Cercosporidium* has seen volatility. Indeed, its author, F.S. Earle, himself vacillated in this regard and the formative nomenclature became rather messy as a result. A year after naming the genus, Earle (1902) had a change of mind, considering *Cercosporidium* to be a synonym of *Passalora*. The binomial *Scolecotrichum euphorbiae* Tracy and Earle (now a facultative synonym of *C. chaetomium* because of the date priority of its basionym, *Cladosporium chaetomium* Cooke) had previously been applied to the type species by Tracy and Earle (1896). Although this was designated as the type of the genus no valid combination into *Cercosporidium* was ever made. However, in a list of synonyms of *Passalora fasciculata* (Cooke & Ell.) Earle [= *Fusicladium fasciculatum* Cooke & Ell.], Earle (1902) listed *Cercosporidium euphorbiae* Earle. Thus the individual who named *Cercosporidium* not only wavered over the status of the name but mistakenly assumed its type species to be identical with another taxon. In reaching the conclusion that the two names were based upon the same anamorph, Earle (1902) was actually following an erroneous determination to this effect made by Shear (1902); this perhaps surprisingly since the conidia of *Cercosporidium chaetomium* and *Passalora fasciculata* are quite a bit different in shape and size. The confusion between these two taxa became longstanding, the distinctiveness between them only being realized following the critical study conducted by Deighton (1967), who maintained the name *F. fasciculatum*. Von Hohnel (1923), interestingly, considered *Cercosporidium* to be distinct from *Passalora* and classified *F. fasciculatum* in the former, as *C. fasciculatum* (Cooke & Ell.) Hohnel. Von Arx (1983) preferred to restrict use of the generic name *Fusicladium* Bon. to anamorphs of *Venturia* Sacc. occurring on Rosaceae, thus advocating continued use of the binomial *Passalora fasciculata*.

Deighton (loc. cit.), following a study of generic types and a comprehensive review of *Cercosporidium*, *Passalora* and some species occurring on leaves of *Euphorbia* L. which he classified in *Fusicladium*, attempted to distinguish, delimit and redefine the respective genera. The former was concluded to be, to quote, "an useful and definable genus, distinct from *Passalora*". His refined concept of these genera was subsequently adopted by Ellis (1971, 1976) but von Arx (loc. cit.) questioned the validity of the separate recognition of *Cercosporidium* and *Passalora* since he considered the type species of the latter, *P. bacilligera* Mont. & Fr., to be "rather similar" to *C. chaetomium*. Von Arx (loc. cit.) reclassified *C. chaetomium* as *Passalora chaetomium* (Cooke) Arx. Sivanesan (1985) expressed a similar opinion that *Cercosporidium* and *Passalora*, as well as *Phaeoisariopsis*, do not differ significantly from one another because of their being based upon what he considered to be variable and unreliable morphological characteristics. The view that these two genera are indistinguishable was subsequently followed by Castañeda and Braun (loc. cit.) and Braun (loc. cit.). Deighton (1990) acknowledged and conceded the similarity existing between the two when he stated that he was "inclined to agree" with the treatment of *Cercosporidium* as synonymous with *Passalora*, although judging from his choice of words it seems reasonable to assume that he nurtured some reservation and uncertainty in this regard. In spite of being doubtful as to whether or not they should remain separate, he apparently remained at least somewhat unconvinced of the desirability of uniting the two genera. In the same publication, Deighton (1990) also lost confidence in the independent status of *Phaeoisariopsis* and expressed doubt as to whether it could be maintained distinct from *Passalora*. He, however, refrained from making any new transfers into the latter genus. Braun (loc. cit.), in contrast, advocated continued separate recognition of *Phaeoisariopsis* as a synnematal counterpart of *Passalora*.

As anamorph form-genera in the *Cercospora*-complex go, *Cercosporidium* appears to the present authors to be as readily definable and easily distinguishable as are a number of others currently recognized. Although there may be a few intermediates in existence which somewhat blur and bridge the morphological divide, a combination of characteristics serve to clearly define *Cercosporidium* and separate it from *Passalora*. The type species of the respective genera, although having some features in common, are quite different in a number of significant respects. The discontinuity between the two is certainly as substantial as is that existing between at least several other genera in this complex. In *Passalora*, a substomatal stroma is either absent or, if present, is usually rudimentary, whereas in *Cercosporidium* it is invariably well-developed, often leading to some disruption of the host tissue as a result of its development. Reflecting the degree of stromal development is the number of conidiophores produced upon each such structure. In *Cercosporidium* a dense fascicle of numerous conidiophores is borne from the stroma. In contrast, species of *Passalora* generally have relatively few conidiophores emerging together in small groups in between host guard cells. Moreover, the growth patterns and certain other details of the conidiophores in the two genera is different. In *Cercosporidium* a tendency for the conidiophores to exhibit incurved growth relative to the overall form of the fascicle is usually pronounced. In addition, the degree of geniculation is often sharply abrupt and conidiogenous loci are frequently widely separated as a result of extensive sympodial proliferation. Associated with this growth pattern is the formation of a thickened band along one side of the conidiophore. This narrow, linear, inequilateral thickening of the conidiophore periclinal wall is similar to that present in the conidiophores of such genera as *Camptomeris* H. Syd. and *Polythrincium* Kunze. Typical conidiophores of *Passalora* are different in that they are more or less straight or somewhat flexuous but not, or only very slightly, geniculate. Conidiogenous loci are generally more gregarious and localized in the distal portion of the conidiophore than is the case in *Cercosporidium*, because of less extensive terminal indeterminate growth, and there is no thickened band formed along their length. In addition, scars at the conidiogenous loci are thickened, conspicuous, and usually prominent in *Cercosporidium* whereas in *Passalora* they are noticeably thinner and less obvious when viewed under the light microscope. The conidial characteristics of these two genera also differ. The conidia of *Passalora* are mostly one-septate, with a swollen, ellipsoidal basal cell subtending a narrower, subcylindric distal cell or cells. In *Cercosporidium* conidia range in shape from cylindrical to obclavate, bear a discernibly thickened proximal hilum, and are, in most species, multiseptate. By consideration of the cumulative combination of these features, sound generic concepts for the respective taxa are assured.

In light of currently accepted generic concepts in this complex, and indeed in the Hyphomycetes in general, together with careful reevaluation of its hallmark characteristics, the original conclusion reached by Deighton to the effect that *Cercosporidium* is a good genus is subscribed to herein. Without unduly belaboring a point, it has to be again said that when it comes to assigning a species to either *Cercosporidium* or *Passalora* there is rarely any difficulty in making a choice. The two genera are sufficiently distinct for there to be very little or no ambivalence in this regard. Although some of the morphological characteristics which distinguish the two may admittedly be variable there are enough consistent features at play for them to be differentiated. In accommodating *Asperisporium sequoiae* in *Cercosporidium* a similar position is being taken to that adopted by Pons and Sutton (1996) when they added *Cercosporidium deightonii* Pons & Sutton to the genus. For the present it certainly seems preferable to keep the genera *Cercosporidium*, *Passalora*, and *Phaeoisariopsis* separate until such time as a more critical and thorough revision of this complex, based on substantive reassessment of criteria, is conducted. Continued redisposition of taxa and inconsistent application of generic concepts without due regard to the respective weight given particular characteristics leads to confusion and contributes little to an improvement in the taxonomy of these anamorphs. Until such time as appropriate discriminating values can be given certain features the taxonomic status quo should be maintained.

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# PESTALOTIOPSIS JESTERI, SP. NOV. AN ENDOPHYTE FROM FRAGRAEA BODENII, A COMMON PLANT IN THE SOUTHERN HIGHLANDS OF PAPUA NEW GUINEA

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## ABSTRACT

*Pestaliotiopsis jesteri,* sp. nov. is described as a coelomycetous endophytic species. It was isolated from the inner bark of *Fragraea bodenii* Wernh. (Gentianaceae) at the singsing grounds of the Aluakambe village in the Southern Highlands of Papua New Guinea. Limb samples taken in the crown, mid-tree and lower levels each yielded *P. jesteri.* This fungus is characterized by the three relatively long appendages arising near the juncture of the conical apical cell and the penultimate cell in the conidium. Each appendage is terminated by a spheroid-like extension. The conidium resembles that of *Pestaliotiopsis distincta* Guba in some respects except for the apices of the three appendages being spheroidal, the long length of the appendages relative to the spore itself and the general fusiform shape of the conidium. Of many plant species studied for their respective endophytes in several locations in the highlands and coastal areas of Papua New Guinea, *P. jesteri* only appeared in one location and in one tree. This finding suggests the host selective nature of this fungus, although it could be readily grown in culture.

**Keywords:** New Guinea, Pestaliotiopsis, appendage apparatus, scanning electron microscopy.

## INTRODUCTION

Endophytic fungi can commonly be isolated from various plant parts. Most often a greater diversity of them occurs from plants growing in tropical rainforests. We have obtained them from stems, petioles, roots, and the main trunk of trees and shrubs representing a wide range of plant families including aquatic ones. Generally, their biological diversity and numbers become reduced as one studies plants ranging closer to either of the earth's poles. One of the most common fungal genera that is isolated as endophytes of tropical plants is *Pestalotiopsis* spp. In fact, this genus is so common, that an examination of our records show that it is exceedingly rare not to find it on the list of endophytes from any tropical plant from any part of the world. Its role in the rainforest ecosystem seems to be unknown, as is its relationship to the plant host.

In an effort to find microbes and their novel biologically active products we search out areas in the world that have large expanses of relatively undisturbed forests that may be hosts to unusual endophytic fungi. Papua New Guinea is on the second largest island in the world and at least 70% of this country is a relatively untouched rainforest. The island is divided east to west by a high ridge of mountains that reach up to 5 thousand meters and are exceedingly rugged. Highland peaks, however, are interrupted by open meadows and valleys. Thus, the enormous difference in elevation gives rise to an equally impressive diversity of flora. In addition, this area is home to at least one million highland people. The Leahy brothers of Australia discovered multiple tribes and clans on a gold hunting expedition in 1930. Their ventures to these lands by foot and by air led to one of the most important anthropological discoveries of the 20th century. Prior to their excursion to this area of New Guinea these people had previously been unknown to the rest of the world. The value, to mankind, of the gold that they recovered in the Papua New Guinea highland streams was replaced by their unexpected discovery and subsequent film documentation of the "first contact" made by the outside world with the native highlanders.

The peoples of Papua New Guinea survive by being extremely knowledgeable of their environment and especially the plant life that grows in almost every geological aspect of it. Scores of plants are used for food, medicines, clothing, structure building, and fire starting sources. Many of these useful plants are native only to Papua New Guinea and/or neighboring Australia since the two land masses were once connected via the York Peninsula during the ice age 10,000 years ago when the ocean waters were shallow or non-existent. Since so many unique plants exist in this part of the world, we reasoned that novel microorganisms could also be present existing in association with them. Thus, with the help of a Huli tribesman we were able to secure a set of samples from a native oak species and study its endophytic fungi. The samples yielded not only a novel endophyte, but a fungus that is novel to fungal taxonomy.

## MATERIALS AND METHODS

Twig samples were taken from *Fragraea bodenii* Wernh. (Gentianaceae), locally referred to as an oak, growing in the Huli valley (ca. 2,000 m elevation) of the Southern Highland Province of Papua New Guinea at coordinates S 05° 04' 00" and E 143° 32' 20". The twigs were about 0.5 m long and 0.3-0.5 cm in diameter. Samples were taken from the crown (25m), the mid-section (12 m) and the lower limbs (5m) of the tree which was located adjacent to the singsing grounds of the Aluakambe village. A local Huli tribesman--Mr. Eric Eroli--kindly scaled the tree and acquired the samples. Until they were processed at Montana State University, the limb samples were held, for the most part, at 4°C. In order to acquire endophytic fungi, the twig pieces were thoroughly soaked with 70% ethanol and the outer bark was removed with a scalpel. Small pieces of whitish inner bark were aseptically removed and placed on a Petri plate containing water agar. Each of the limb samples had twelve pieces of inner bark removed and four pieces placed on each of three Petri plates. After incubation for several days, hyphal tips of developing fungi were aseptically removed and placed on potato dextrose agar (PDA). Pure fungal cultures were obtained in this manner. After 10 days fungal colonies were transferred to gamma irradiated carnation leaves (0.5x 0.5 cm) to encourage spore production.

Carnation leaves supporting fungal growth and acervular production were placed into distilled water with Triton x 100 (wetting agent) and aspirated for 5 min. The fungal preparation was then placed into a 2% glutaraldehyde in 0.1 M sodium calodylade buffer (pH 7.2-7.4) and left overnight. The next day they were washed in six 15 min changes of water buffer (1:1, v:v), followed by three 15 min changes in 10% ethanol, four 15 min changes of 30% ethanol, five 15 min changes of 50 % ethanol and left for two days in 70% ethanol. They were then rinsed in five 15 min changes of 95% ethanol and five 15 min changes in 100% ethanol. The dehydration process was slowly done to discourage the process of spore shriveling which occurs during rapid dehydration. The fungal material was critically point dried, gold sputter coated and examined with a JEOL 6100 scanning electron microscope. Laser scanning light micrographs were taken with a Zeiss confocal microscope equipped with an argon laser with 488 and 514 wavelengths.

## RESULTS AND DISCUSSION

### Oak Endophytes

With the help of a native Huli tribesman, Eric Eroli, who scaled a *F. bodenii* tree, twig samples throughout the crown of the tree were recovered (Fig.1). From the top of the crown of the tree 18 endophytic fungi were recovered. Of these, ten microbes answered the general description of *Pestalotiopsis microspora* (Strobel et al., 1996) and six fungi did not produce fruiting structures and thus remain unknown. However, one fungus 6T-l-3, the topic of this report, appeared as a novel pestalotiopsis species while the remaining one also appeared as a novel pestalotiopsis and will be the subject of another report. From the mid-section of the crown of the oak tree at the 12m level, 16 endopyhtic fungi were recovered. Eight of these were tentatively identified as *Pestalotiopsis microspora*, five unknowns, and one pycnidial producing fungus. Also appearing was one unknown pestalotiopsis-like fungus and 6M-B-3, which was morphologically identical to 6T-1-3 (above). The limb from the bottom of the tree yielded four *P. microspora* isolates, one pycnidial forming fungus, four unknown fungi, two unknown pestalotiopsis species, and 6B-S-4 which was morphologically identical to 6T-1-3 and 6T-B-3 (above). Thus, the upper parts of this one representative tree (*F. bodenii*) are serving to support a wide range of endophytic fungi with the most predominate fungal genus being *Pestalotiopsis* sp. While the majority of these isolates resembled *P. microspora,* there were several collected that appeared to be new species of this genus and one in particular (6T-1-3, 6B-S-4, and 6M-B-4) is the subject of this report.

## Fungal Taxonomy

***Pestalotiopsis jesteri*** Strobel, Li, Ford, and Hess sp. nov. (Fig. 2-8).

***Fungus**, in foliis Dianthi caryophylli cretis, acervulis quibus sunt erumpentibus pustuliformibus unilocularibus et ovalibus ad irregularibus in ambitu (Fig. 2). **Acervuli** plerumque 100-200 μm diametro, aliquando ad paene 300 μm. **Dehiscentia** ope fissurae rectiusculae simplicis ad partem apicalem acervuli sic sporae liberantes (Figs. 3 & 4). **Conidiophora** in pariete interiore integra acervuli orientia. **Conidia** fusiformia, quadriseptata, plerumque sine constrictionibus ad septa, 19-23 × 5-7 μm, medio 19 × 6 μm (Fig. 5), et appendicibus inramosis ferentia, appendice ad cellulam basalem 3-8 μm longitudine (4.5 μm mediante) et appendicibus 3 (raro 4) ad juncturam cellulae apicalis conicae orientibus. Cellula conidica paenultima in sporis maturis appendicibus 11-28 μm longitudine (23 μm mediantibus; Fig. 6). **Appendices** 0.6-1.0 μm diametro unaquaeque apice globoso ca 1.5 μm diametro mediante (Fig. 7). **Cellula apicalis conica** ca. 3.0 μm longitudine medians. Cellulae et apicales et basales hyalinae autem cellulae centrales tres brunneae ad pallidibrunneae et sine characteribus distinguibilibus ullialiis (Fig. 8).*

**Fungus**, grown on carnation leaves, with acervuli which are erumpent, pustule-like, unilocular, and oval to irregular in outline (Fig. 2). **Acervuli** generally 100-200 μm in diameter, occasionally up to nearly 300 μm. **Dehiscence** by means of a simple, fairly straight split in the apical region of the acervulus thus releasing the spores (Figs. 3 & 4). **Conidiophores** arising on the entire inner acervular wall. **Conidia** fusiform, 4 septate, usually without constrictions at the septa, 19-23 × 5-7 μm, with the average 19 × 6 μm (Fig. 5), and bearing unbranched appendages, with the appendage at the basal cell 3-8 μm in length (averaging 4.5 μm) and with 3 (rarely 4) appendages arising at the juncture of the conical apical cell. Penultimate conidial cell on mature spores with appendages 11-28 μm in length (averaging 23 μm; Fig. 6). **Appendages** 0.6-1.0 μm in diameter, each one with a spherical tip averaging ca. 1.5 μm in diameter (Fig. 7). **Conical apical cell** averaging ca. 3.0 μm in length. Both the apical and basal cells hyaline but the 3 central cells brown to pale brown and without any other distinguishing characters (Fig. 8).

**Holotypus:** Endophytic on *Fragraea bodenii* in Papua New Guinea. Specimen collecting was done by Gary Strobel with the help of Eric Eroli, a Huli tribesman. The fungus (three isolates) is deposited in the Montana State University Herbarium (MONT) as Strobel 6T-L-3, 6M-B-3, and 6B-S-4. Living cultures under the same designation are in the Montana State University Mycological collection.

**Telomorph:** Unknown

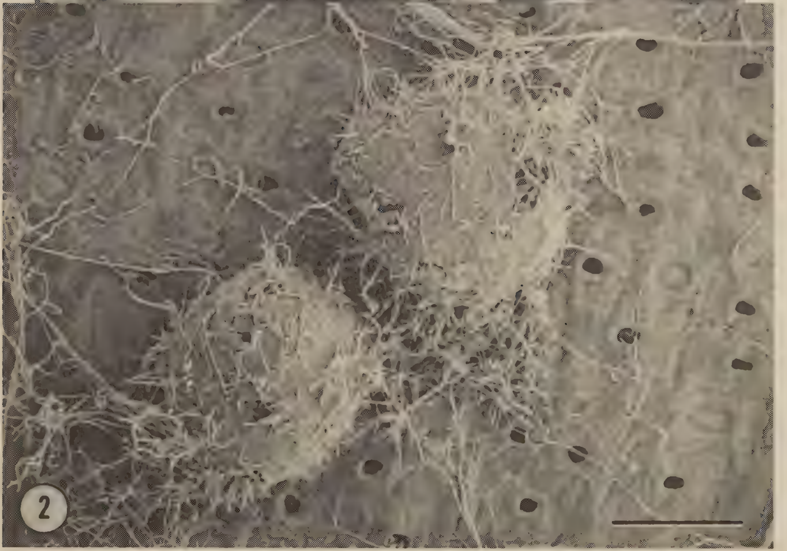
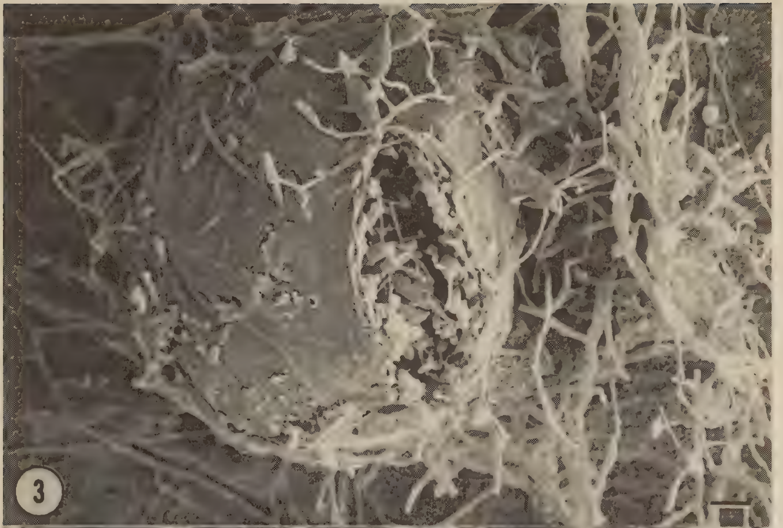


Fig. 1. New Guinea Huli tribesman securing twig samples from an oak tree.
Fig. 2. A scanning electron micrograph of *P. jesteri* acervuli. Bar=100 μm.

## Comparative Taxonomy

While three isolates of *P. jesteri*, obtained from totally different locations in the same oak tree in Papua New Guinea, possessed identical spore and acervular morphologies, they appeared to be different culturally and biochemically. For instance, in 6T-L-3, the margin of the rapidly growing colony on PDA was even, entire, and circular with a light coloration. In isolates 6M-B-3 and 6B-S-4 the colonies were slow growing with irregular margins and possessing a solid pinkish coloration. The later two isolates, differing somewhat culturally, both produced a novel cyclic compound with multiple quaternary carbons, carbonyl and hydroxyl functionalities. This product will be the subject of another report. However, isolate 6T-L-3 did not produce this compound but a series of other substances that seem to be unique to it. In addition, while 18S rDNA analysis of *P. jesteri* demonstrated more than 98% analogy to that of *P. microspora*, some small regions contained sequence differences (Worapong, unpublished). These genetic analyses further showed evidence of relatedness of *P. jesteri* to the pestalotiopsis group. It does seem to be the case that various species of pestalotiopsis possess enormous genetic variation and our observations on *P. microspora* from bald cypress further substainate this generalization (Li et al., 1996). While it is common to isolate pestalotiopsis from virtually every rainforest plant, the isolates may appear identical morphologically, but culturally and biochemically be drastically different (Li et al., 1996). The explanations for this may, in part, reside with the relative ease in which pestalotiopsis is genetically modified (Long et al., 1998). Thus, since it is endophytic in many plant species, it may be able to acquire multiple genetic traits and use them to its selective advantage. The fungus is also readily mutable by UV radiation (Ford and Strobel, unpublished). Thus, when it grows on leaf surfaces in rainforest canopies exposed to the sun's rays, mutants may ultimately be produced.

While literally hundreds of species of pestalotiopsis have been described most are categorized on the basis of the host plant from which they were obtained (Raj, 1993). A close examination of the described acervular and spore morphologies of these organisms reveals that they are identical to *P. microspora* (Sutton, 1980). This is illustrated, for instance, by the descriptions of seemingly new pestalotiopsis species but both have morphological identity to *P. microspora* (Sati and Tiwari, 1993; Pal and Purkayastha, 1992). However, given the general trend of naming more and more species of pestalotiopsis on the basis of the plant host from which they may be obtained, it would seem likely that there is no end in sight to the species of this fungus that may continue to be described. However in our case, a comprehensive computer based literature search revealed that no recently described pestalotiopsis has been reported having identity to *P. jesteri*. In addition, genetic analysis using 18S rDNA techniques strongly indicated that our organism belongs to the genus *Pestalotiopsis* (Worapong, unpublished). In addition, *P. jesteri* has many general morphological characteristics resembling or equivalent to those in the genus pestalotiopsis (Sutton, 1980 and Raj, 1993). However, the fungus in this report seems to be distinct from all other species in this genus by several important morphological characteristics. It differs from *Pestalotiopisis distincta*, which appears to be its most closely related taxonomic type, mainly by acervular and conidial features. Although the conidia of *P. distincta* possess generally 3 apical



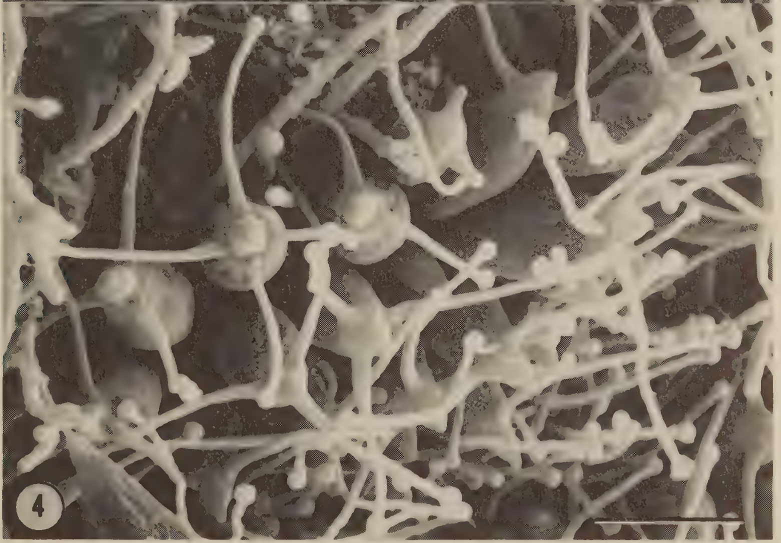
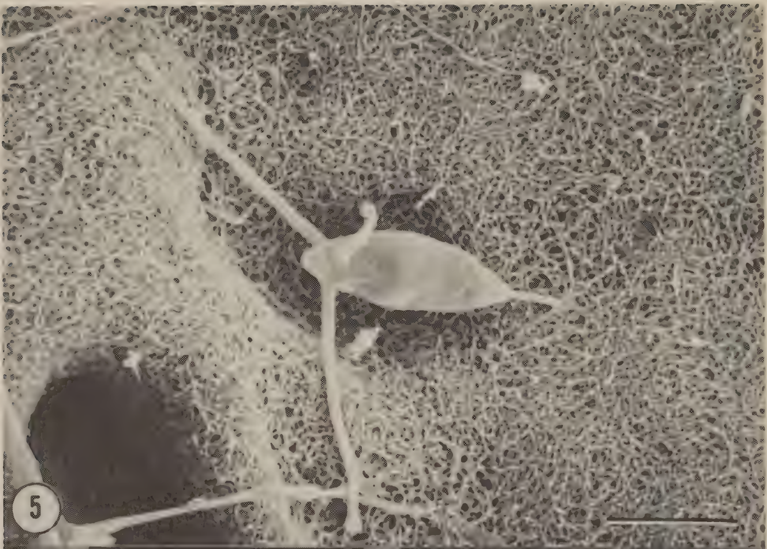


Fig. 3. A scanning electron micrograph of a dehiscent *P. jesteri* acervulus showing extruding spores. Bar=10 µm.

Fig. 4. A scanning electron micrograph of developing *P. jesteri* conidia. The top of the acervulus was removed. Bar=10 µm.



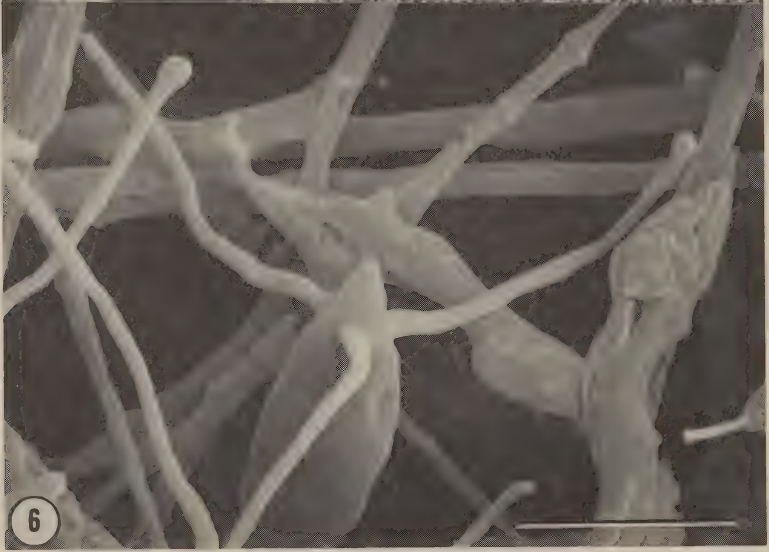
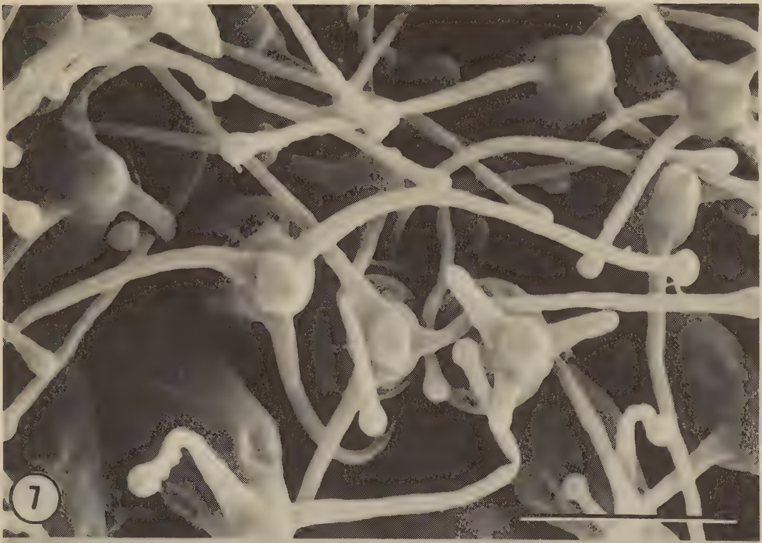


Fig. 5. A scanning electron micrograph of a *P. jesteri* conidium showing the characteristic three appendages. Bar=10 µm.

Fig. 6. A scanning electron micrograph of a *P. jesteri* conidium showing attachment of appendages. Bar=10 µm.



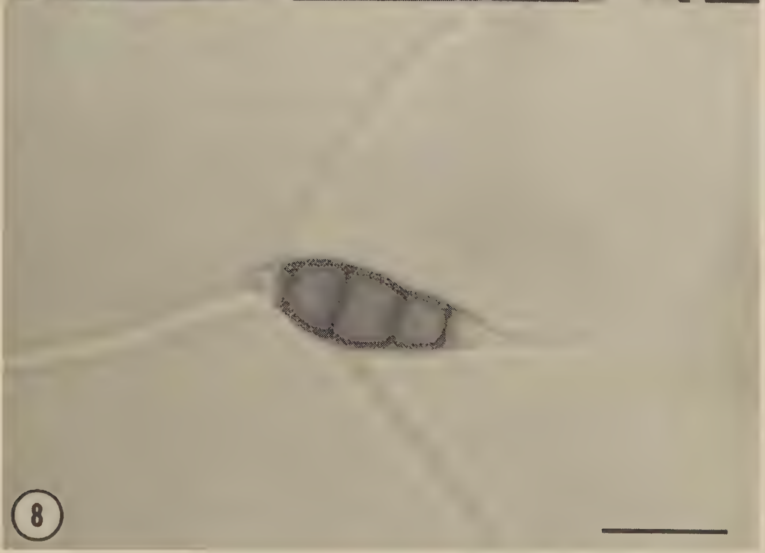


Fig. 7. A scanning electron micrograph of *P. jesteri* conidia showing the characteristic spheroids at the tips of the appendages. Bar=10 µm.

Fig. 8. A laser scanning light micrograph of a *P. jesteri* conidium showing the hyaline apical and basal cells and darker coloration of the three center cells.
Bar=10 µm.

appendages arising from the base of the conical apical cell, they have no terminal enlargements (spheroids) on the appendages and these are much shorter (relative to the length of the cell) than in the case of *P. jesteri* (Raj, 1993). In addition, the acervuli of *P. dictincta* are generally larger than *P. jesteri.* Dehiscence of the acervulus of *P. dictinta* occurs in an irregular manner versus a simple straight line of dehiscence in the acervulus of *P. jesteri.* Furthermore, the basal cells of the conidia of *P. distincta* are obconic, while in *P. jesteri* the basal conidial cells are conical.

## ACKNOWLEDGEMENTS

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# MACROLEPIOTA GLOBOSA ET NOTHOPANUS NSIMALENENSIS, DEUX NOUVELLES ESPÈCES FONGIQUES DU CAMEROUN

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**Résumé**. Deux nouvelles espèces de champignons lamellés (Basidiomycètes) sont décrites du Cameroun. *Macrolepiota globosa* sp. nov. semble proche de *M. odorata* mais en diffère, entre autres, par son chapeau restant subglobuleux et sa sporée jaune à jaune-ochracé. *Nothopanus nsimalenensis* sp. nov. ressemble à *N. hygrophanus* mais possède un système d'hyphes dimitique.

**Mots-clés** : *Macrolepiota* (Agaricaceae), *Nothopanus* (Tricholomataceae), Basidiomycotina

**Abstract** : Two new species of gilled fungi (Basidiomycetes) are described from Cameroon. *Macrolepiota globosa* sp. nov. seems rather close to *M. odorata* but differs mainly in its cap remaining subglobose and its yellow to ochraceous yellow spore-print. *Nothopanus nsimalenensis* sp. nov. looks like *N. hygrophanus* but shows a dimitic hyphal system.

**Key-words** : *Macrolepiota* (Agaricaceae), *Nothopanus* (Tricholomataceae), Basidiomycotina

## INTRODUCTION

La flore mycologique du Cameroun et de la plupart des pays d'Afrique au sud du sahara, poutant très riche et diversifiée, est jusqu'à ce jour plutôt très mal connue contrairement par exemple à celle des végétaux supérieurs. A part quelques rares études parcellaires faites çà et là par quelques auteurs isolés, et cela généralement depuis plusieurs dizaines d'années, il n'existe à ce jour à proprement parlé aucun document sur la flore des champignons du Cameroun, les seules études fiables effectuées dans la zone de l'Afrique centrale ayant été faites essentiellement sur la région des grands lacs de cette partie de l'Afrique. C'est pour essayer de combler ce vide qu'une vaste étude taxonomique de la flore des macromycètes du Cameroun a été entreprise avec pour point focal les Agaricales et les Polyporales. Les espèces nouvelles ici décrites se situent dans le cadre de ce travail dont le but ultime est de dresser un "Check list" et une carte aussi complète que possible des espèces de macromycètes poussant au Cameroun.

## MATÉRIELS ET MÉTHODES

Les spécimens ramenés des collectes sur le terrain sont séchés sur un séchoir électrique de type DÖRREX après que les caractères changeant ou disparaissant lors du séchage aient été au préalable notés sur le terrain ou peu avant le séchage ou alors par la suite sur les photos ou diapositives couleur prises sur le terrain avant la récolte. Les spécimens sont ensuite étiquetés et conservés dans "l'herbier D.C. Mossebo" rattaché à celui du Département de Biologie et Physiologie végétales (Laboratoire de Cryptogamie) de l'Université de Yaoundé 1.

L'étude microscopique des échantillons est effectuée sur coupes extra-minces observées au microscope à lumière électrique après les colorations usuelles. Les colorants et les réactifs utilisés sont les suivants : du KOH (10%) uniquement pour regonfler les exsiccata ou alors de la Glycérine à 10% pour les spécimens frais, de la Phloxine B et du Rouge Congo pour la coloration des spores et des structures hyméniales, le réactif de Melzer pour déterminer l'amyloïdie ou la dextrinoïdie des spores et du bleu de crésyl pour déterminer leur métachromasie. La teinture de Gaïac, le Gaïacol, le Pyrogallol, le α-Naphtol et l'Ammoniaque ont été également utilisés pour les réactions colorées de la chair de *Macrolepiota globosa*, le teinture de Gaïac ayant aussi servi pour déterminer le type de pourriture causé au bois par *Nothopanus nsimalenensis*.

### *MACROLEPIOTA GLOBOSA* SP. NOV. : Figure n° 1

Quelques basidiomes appartenant au genre *Macrolepiota* Singer furent collectés en novembre 1996 sur le campus de l'Université de Yaoundé 1 (capitale) au Cameroun. Leur examen macroscopique et microscopique montra qu'ils présentaient certains caractères particuliers qui ne furent retrouvés chez aucune autre espèce de ce genre. D'autres basidiomes beaucoup plus grands furent, par la suite (août 1999), récoltés près d'un champ d'ignames aux environs de la ville de Yaoundé. Ils présentaient les mêmes caractéristiques que la première récolte. L'espèce est donc proposée ici comme nouvelle.

***Macrolepiota globosa*** Mossebo sp. nov. Fig. 1

***Pileus*** *5-12 cm latus, ovoideus vel subglobosus in juventute, globosus, interdum subsemirotundus, non expansus in maturitate, pelliculosus, albidus, a squamis brunneis totaliter tectus, in margine striatus.* ***Cuticula*** *ex hyphis vallatis, septatis, agglutinatis, tenuitunicatis, nonnullis apicaliter parve obtusis.* ***Stipes*** *10-28 x 1-2,5 cm, albidus, cylindricus, ad basim bulbosus, cavus, leviter rubescens ad sectionem.* ***Annulus*** *duplex, 5-7 mm latus, mobilis.* ***Caro*** *albida, leviter rubescens ad sectionem, glauca in guiaco gummi, purpurea in guiacolo, pallide brunnea in pyrogallolo , sine reactione in α-naphtolo et ammonio.* ***Lamellae et lamellulae*** *albidae, rectae vel interdum sinuosae, liberae, densae.* ***Trama hymenophoralis*** *irregularis vel intricata.* ***Subhymenium*** *pseudoparenchymatosum.* ***Basidia*** *15-22 x 7-8 µm, clavata, 4-sporigera.* ***Cheilocystidia*** *27-35 x 16-20 µm, clavata vel piriformia, tenuitunicata, hyalina.* ***Pleurocystidia*** *nulla.* ***Sporae*** *8-11 x 5-6 (7) µm, isabellinae vel subflavae, late ovales vel ellipsoideae, truncatae cum poro germinativo, crassitunicatae atque stratificatae, cum una crassa oleosa guttula solum in novis sporis, dextrinoideae metachromaticae.* ***Depositum sporarum*** *flavum vel flavo-ochraceum.* ***Habitatio*** *solitarie ad humum herbosum in umbra.* ***Holotypus****:"D.C.Mossebo 98-1", in Herbario Universitatis Yaoundeae, ad*

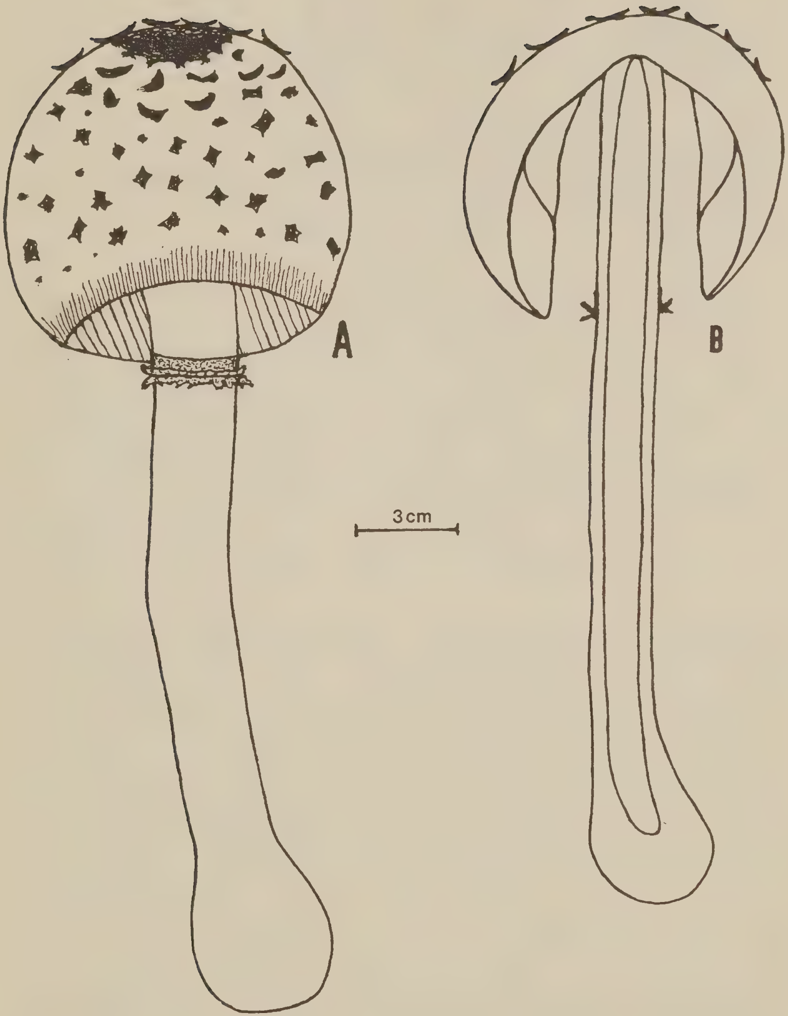

**Fig. 1A-B** : *Macrolepiota globosa* (*Mossebo* 98-1). **A**- Basidiocarpe entier.
**B**- Basidiocarpe en coupe longitudinale

**Fig. 1 (suite)**

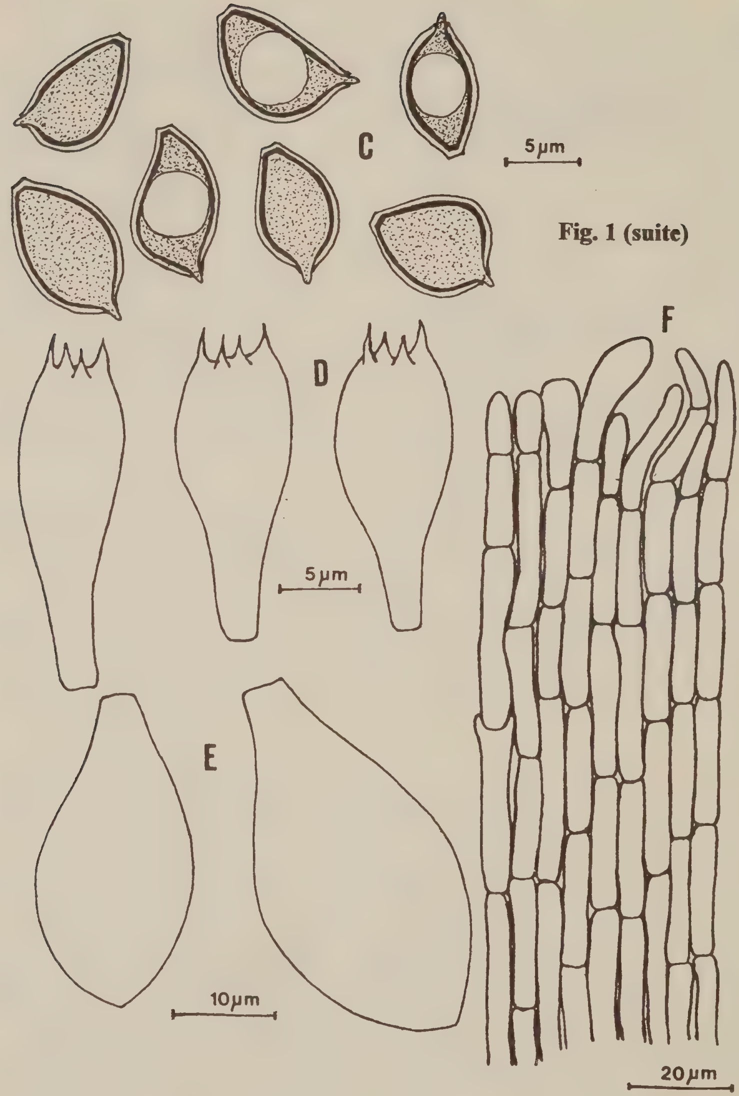

**Fig. 1C-F** : **C**- Basidiospores. **D**- Basides. **E**- Cheilocystides. **F**- Cellules de l'épicutis

*humum herbosum sub arbore in campo Universitatis, 780 m altitudine, Yaounde,Camerun, 1 novembre 1996, D.C. Mossebo.*

### Description macroscopique.

**Chapeau** 5-12 cm de diamètre, ovoïde à subglobuleux à l'état jeune, puis globuleux ou subhémisphérique à maturité, longtemps lié au pied par l'anneau, s'ouvrant à peine mais ne s'étalant pas à l'état mûr, mince à pelliculaire, strié à la marge par la marque des lamelles, blanchâtre, presque entièrement couvert de squames brun-marron adhérentes à l'état jeune, se détachant en squamules sur basidiomes mûrs en laissant au centre une plaque squameuse brunâtre de 1,5-3 cm de diamètre. **Stipe** 10-28 cm x 1-2,5 cm, blanc, droit, parfois légèrement tordu, à surface glabrescente à légèrement fibrilleuse, bulbeux à la base avec un bulbe pouvant mesurer jusqu'à 3-4 cm de diamètre sur pieds adultes, creux et rougissant légèrement sur la surface de coupe. **Anneau** double, 5-7 mm de large, mobile. **Lamelles** 0,5 à 1,5 cm de large sur carpophores adultes, blanches, libres, parfois légèrement sinueuses, serrées avec présence de lamellules, à arêtes subrégulières, souvent finement bordées de minuscules taches noirâtres discontinues visibles tout le long de l'arête. **Sporée** jaune à l'état frais, changeant en jaune ochracé sur la durée lors de la conservation. **Chair** blanche, 0,5 à 1,5 cm d'épaisseur au contact du pied, rougissant également à la coupe, mais plus légèrement que le stipe. **Réactions colorées de la chair** : *Teinture de Gaïac* : bleu-verdâtre instantané. *Gaïacol* : pourpre à rosé faible et réaction lente. *Pyrogallol* : marron clair. *α-Naphtol*: réaction nulle. *Ammoniaque*: réaction nulle. **Odeur** de parfum anisé.
**Habitat** : terrestre poussant à l'ombre dans la litière sur sol herbeux. **Croissance** isolée, mais en petits groupes.
**Distribution** : récolté seulement au Cameroun sur les lieux décrits.

### Description microscopique.

**Spores** 8-11 x 5-6 (7,5) µm, beiges à jaunâtres, largement ovales à ellipsoïdes avec un pore germinatif (≈ 2 µm) tronquant légèrement l'apex, à paroi épaisse et stratifiée, métachromatique avec l'endospore plutôt violacée dans le bleu de crésyl, dextrinoïdes, présentant une grosse guttule (essentiellement sur spores jeunes) qui se disperse assez rapidement. **Basides** 15-22 x 7-8 µm, largement clavées, à 4 stérigmates, bouclées à la base. **Cheilocystides** 27-35 x 16-20 µm, nombreuses, hyalines à légèrement pigmentées, largement clavées à piriformes et à paroi mince. **Pleurocystides** absentes. **Trame de l'hyménium** d'apparence plutôt irrégulière à emmêlée (± dense et touffue). **Sous-hyménium** pseudoparenchymateux. **Suprapellis** hyméniforme, constitué d'hyphes cloisonnées (120-160 x 4-6 µm) agglutinées, à paroi mince, la plupart arrondies, certaines obtuses.

### Récoltes.

**Holotype** : Cameroun, Yaoundé, sur litière au sol dans le gazon à l'ombre d'un arbre sur le campus de l'Université de Yaoundé 1, altitude 780 m, 01 Novembre 1996, collection D. C. Mossebo : "D. C. Mossebo 98-1" (Herbier du Département de Biologie et Physiologie végétales rattaché au Laboratoire de Cryptogamie de l'Université de Yaoundé 1)

**Paratype** : Cameroun, environs de Yaoundé près d'une plantation d'ignames (*Discorea alata*), altitude 790 m, 25.08.1999, collection D.C. Mossebo: "D.C. Mossebo 98-3" (même Herbier que le Holotype)

**Commentaires.**

Deux caractères principaux sont diagnostiques de *Macrolepiota globosa*. D'une part la forme du chapeau qui reste plus ou moins globuleux à subhémisphérique et fixé au pied par l'anneau jusqu'à l'état adulte, ne s'en détachant qu'au stade ultime de son développement sans toutefois s'étaler. D'autre part la couleur jaune à jaune ochracé de sa sporée est exeptionnelle chez les *Macrolepiota*.

L'espèce dont elle se rapproche le plus, par certains de ses caractères macroscopiques et microscopiques, est *Macrolepiota odorata* décrite par Heinemann (1969, 1970), mais elle en diffère par :

- son chapeau un peu plus grand et restant globuleux à subhémisphérique, sans mamelon chez *M. globosa*; plutôt globuleux (4-10 cm de diamètre), "largement et bassement mameloné" chez *M. odorata* où il s'ouvre un peu plus à maturité,
- sa sporée de couleur jaune à jaune ochracé chez *M. globosa*, blanc pur chez *M. odorata,*
- son anneau, double chez *M. globosa*, plutôt épais et étalé horizontalement chez *M. odorata,*
- son stipe en général un peu plus long et épais et non teinté chez *M. globosa,* "teinté par places de bleu verdâtre pâle" chez *M. odorata* où il mesure 7-16 x 0,5-1,3 cm,
- ses basides uniquement tétrasporiques chez *M. globosa,* "(2) - 4 spores chez *M. odorata*" où elles mesurent 24 x 7 µm,
- la trame de l'hyménium plutôt irrégulière à emmêlée chez *M. globosa*, caractère exceptionnel chez les *Macrolepiota* où la trame est plutôt généralement régulière et enfin
- la différence de sensibilité à certains réactifs chimiques dont particulièrement le α-Naphtol, le Gaïacol et le pyrogallol. En effet, la chair de *M. odorata* est immédiatement violet intense dans le α-Naphtol, immédiatement pourpre intense dans le Gaïacol et lentement brun chocolat dans le pyrogallol.

**Discussion.**

*Macrolepiota globosa* étant une espèce nouvelle, il est nécessaire de rechercher sa position systématique par rapport aux récents systèmes de classification, dont notamment ceux de Singer (1986) et de Bon (1996). Concernant les *Macrolepiota*, Singer (1986) met l'accent sur la présence ou l'absence de boucles; il s'est avéré par la suite que ce système posait des problèmes en fonction de la discrétion ou de la netteté même de ces boucles, quelles que soient les techniques de coloration et d'observation utilisées. En effet, en dehors de quelques espèces, dont par exemple *Macrolepiota africana* (Heim) Heinem, *M. gracilenta* (Kromb.) et *M. zeyheri* (Berk.) Singer, dont les boucles ont été clairement observées et décrites, les rendant ainsi faciles à classer dans le système de Singer, la position réelle de plusieurs autres espèces reste incertaine, la présence ou l'absence de boucles n'ayant été signalée par les auteurs en raison sans doute des difficultés d'appréciation sus-mentionnées. C'est le cas par exemple chez *Macrolepiota odorata* Heinem. et *M. rhacodes* (Vitt.) Singer. ou encore chez *M. procera* (Scop. : Fr.) Singer et *M. mastoidea* (Fr.) Singer, où les boucles sont incertaines car à peine visibles ou disparaissant lors de la maturation des basidiomes. En conséquence de ces difficultés, Bon (1996) a proposé un autre système de classification des *Macrolepiota*, basé sur d'autres caractères distinctifs, par exemple le caractère elliptique ou non des spores, leur taille ainsi que la présence ou non d'un cal lenticulaire proéminent.

*Macrolepiota globosa*, avec ses boucles plutôt bien apparentes à la base des basides, appartiendrait au groupe des espèces bouclées, dans ce système de Singer (1986). Dans le système de Bon (1986), *M. globosa* semblerait se rapprocher de la section *Laevistipedes* (Pazmany) M. Bon, étant donné la taille de ses spores, qui ne sont par ailleurs pas parfaitement elliptiques, et l'absence de cal lenticulaire proéminent. Cependant, l'espèce s'en éloigne également par la couleur jaune à jaune ochracé de sa sporée, caractère exceptionnel dans ce genre. Ce dernier critère pose d'ailleurs le problème de la nécessité éventuelle de créer une nouvelle section (ou peut-être une sous-section de la section *Leavistipides*), qui s'identifierait principalement à cette couleur de la sporée. De nouvelles récoltes de notre taxon et peut-être la découverte d'autres espèces possédant ce caractère remarquable permettront d'apporter, dans l'avenir, des éléments de réponse à cette intéressante question.

### *NOTHOPANUS NSIMALENENSIS* SP. NOV. : Figure n° 2

Des basidiomes blanchâtres, pleurotoïdes furent récoltés en octobre 1997 sur des troncs en dégradation au sol dans une plantation de bananiers située dans la banlieue de Yaoundé au Cameroun, près de l'aéroport international de Nsimalen. D'autres furent récoltés par la suite (août 1998) dans une plantation de caféiers au lieu dit “Banla'a” près de Nkongsamba-ville à près de 420 km au nord-ouest de Yaoundé. Leur examen macroscopique et microscopique a permis de les classer dans le genre *Nothopanus* Singer, mais certains caractères ne coïncidant avec ceux d'aucune espèce du genre, l'espèce est proposée ici comme nouvelle.

***Nothopanus nsimalenensis*** Mossebo sp. nov. Fig. 2
***Pileus*** *5-7 x 3-4 cm latus, reniformis vel flabelliformis, flexibilis, albidus, fusco-aurantiacus ad stipem, cum sparsis obscuris vel nigrescentibus maculis, striatus ad marginem.* ***Cuticula*** *hymeniformis, 30-60 μm crassa, cellulis vallatis basidiiformibus 10-18 x 3-5 μm, saepe apicaliter inflatis et digitatis.* ***Caro*** *tenuissima vel membranacea, albida.* ***Stipes*** *5-12 x 4-5 mm, curtus, cylindricus, albidus, glaber, generaliter lateralis, raro excentricus.* ***Lamellae et lamellulae*** *e pileo concolor, rectae, regulares, distantes, leviter decurrentes.* ***Trama hymenophoralis*** *subregularis vel intricata, dimitica cum hyphis generativis fibulatis, 5-8 vel 10-12 μm latis et hyphis sceletalibus 5-15 μm latis, hyphis gelatinosis inflatis 25-86 x 17-20 μm praesentibus.* ***Subhymenium*** *pseudoparenchymatosum.* ***Basidia*** *15-17 x 4-6 μm, cylindrica, interdum clavata, 2-4-sporifera, 3-4 μm longis sterigmatibus.* ***Cheilocystidia*** *nulla.* ***Pleurocystidia*** *20-25 x 7-9 μm, rara, lageniformia vel utriformia, raro cylindrica.* ***Sporae*** *5-6 x 2-3 μm, hyalinae, ellipsoideae, tenuitunicatae.* ***Depositum sporarum*** *album.* ***Habitat*** *in ligno.* ***Ligni putrefactio*** *brunnea, cuboidea.*
***Holotypus***: *“D.C.Mossebo 55-B” in herbario Universitatis Yaoundeae, ad putrescentem deciduum truncum arboris, in musarum campo, Nsimalen, prope Yaounde, Camerun, 14 october 1997, D.C. Mossebo.*

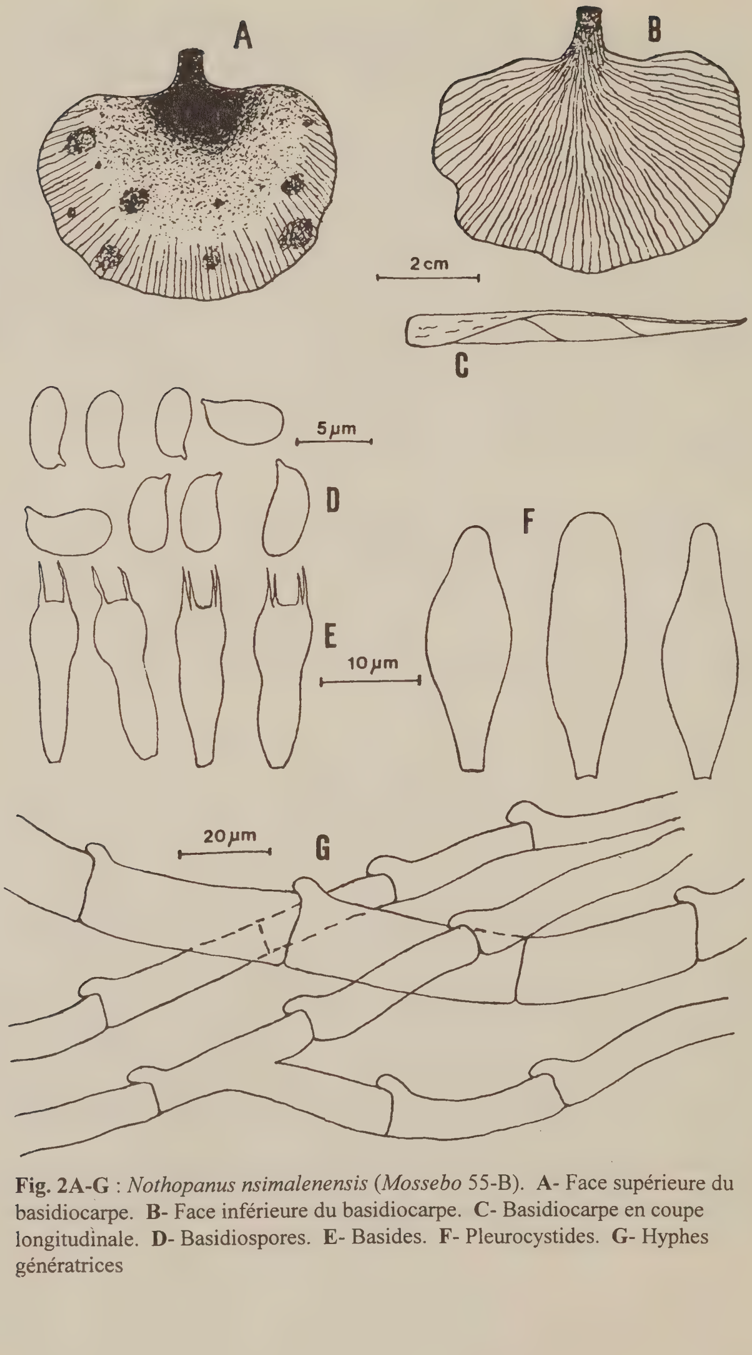

**Fig. 2A-G** : *Nothopanus nsimalenensis* (*Mossebo* 55-B). **A**- Face supérieure du basidiocarpe. **B**- Face inférieure du basidiocarpe. **C**- Basidiocarpe en coupe longitudinale. **D**- Basidiospores. **E**- Basides. **F**- Pleurocystides. **G**- Hyphes génératrices

Fig. 2 (suite)

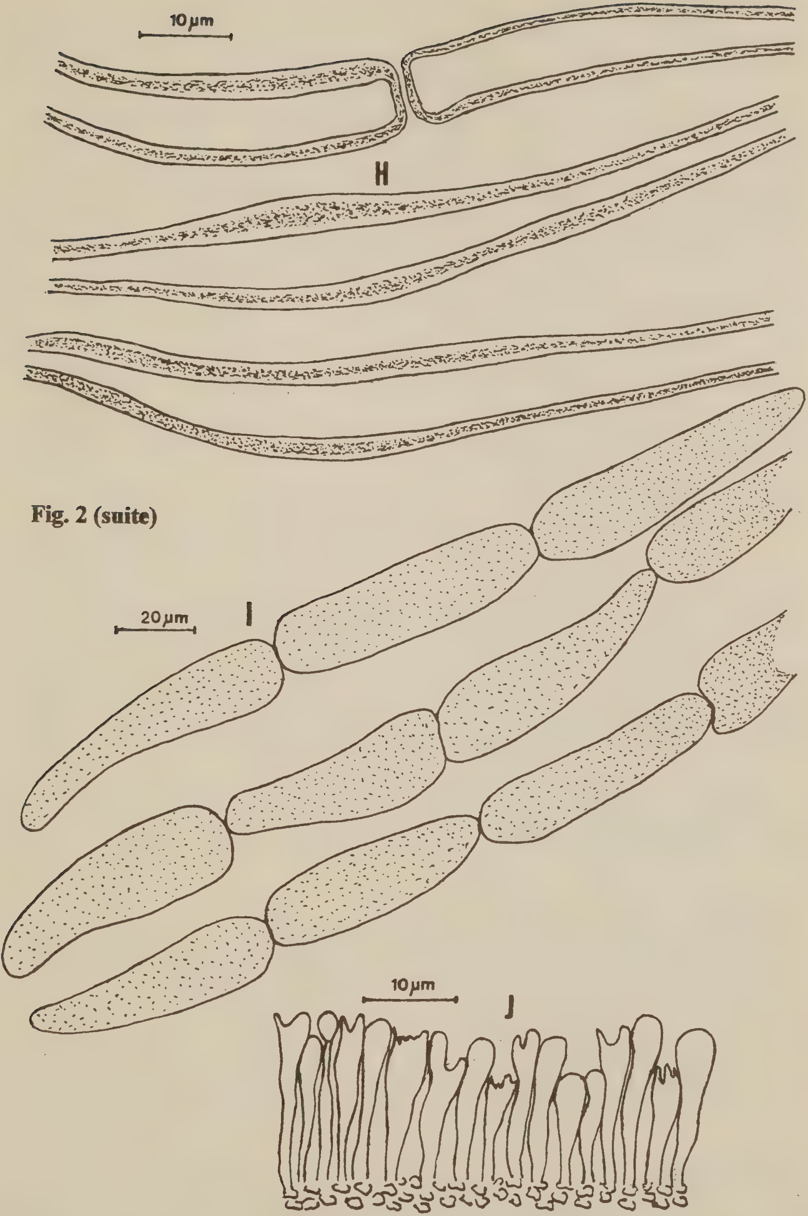

**Fig. 2H-J**: **H**- Hyphes squelettiques. **I**- Hyphes gélatinisées. **J**- **Cellules de l'épicutis**

### Description macroscopique.

**Chapeau** réniforme à flabelliforme, 5-7 cm de diamètre ou 5-7 x 3-4 cm à maturité, blanchâtre avec une zone d'un brun orangé plus ou moins vif près de l'attache avec le stipe et de petites taches sombres à noirâtres plus ou moins éparses en surface, souple, glabre, présentant des sillons de longueur variable à la marge, correspondant à la disposition des lames et lamelles. **Stipe** court, cylindrique, 5-12 x 4-5 mm, plein, blanchâtre, glabre, généralement latéral, rarement excentrique. **Lamelles** blanchâtres, concolores avec le chapeau, 3-4 mm de large, horizontales, à arête régulière, relativement espacées et légèrement décurrentes; présence de lamellules de plusieurs dimensions. **Sporée** blanche. **Chair** très mince à membraneuse, < 1-2 mm d'épaisseur, blanchâtre avec souvent par endroits des taches marron sombre à noirâtres, se prolongeant dans la trame de l'hyménium dont elle a les mêmes caractéristiques microscopiques. **Odeur** agréable.

**Habitat** : lignicole, poussant sur bois mort humide. **Type de croissance** : très souvent imbriquée par leurs stipes, rarement isolée. **Type de pourriture** : brune, réaction négative à la teinture de Gaïac.

**Distribution** : Récolté jusqu'ici uniquement au Cameroun dans la région sud (à Yaoundé) et littorale à Nkongsamba.

### Description microscopique.

**Spores** 5-6 x 2-3 µm, hyalines, ellipsoïdes, à paroi mince, non amyloïdes. **Basides** 15-17 x 4-6 µm, parfois clavées, très souvent cylindracées, bi- ou tétrasporiques avec de longs stérigmates d'environ 3-4 µm. **Cheilocystides** absentes. **Pleurocystides** 20-25 x 7-9 µm, très rares, lagéniformes à utriformes, rarement cylindracées et rétrécies à leur partie inférieure. **Trame de l'hyménium** subrégulière à emmêlée, à système d'hyphes dimitique avec des hyphes génératives bouclées de deux diamètres (Ø = 5-8 µm et 10-12 µm) et des hyphes squelettiques de 5-15 µm de diamètre. Présence également d'hyphes gélatinisées enflées et à contenu réfringent, de longueur variable (25-86 µm) et d'une largeur de 17-20 µm. **Sous-hyménium** pseudoparenchymateux avec des hyphes de 5-8 µm de diamètre. **Suprapellis** de 30-60 µm d'épaisseur, hyméniforme, à cellules mesurant 10-18 x 3-5 µm, étroitement serrées les unes contre autres avec sommets multiformes généralement élargis et présentant très souvent de minuscules ramifications.

### Récoltes.

**Holotype** : Cameroun, près de l'aéroport international de Nsimalen à 20 km de Yaoundé, sur tronc d'arbre en dégradation au sol dans une plantation de bananiers, 14.10.1997, collection D.C. Mossebo : "D.C.Mossebo 55-B" (Herbier du Département de Biologie et Physiologie végétales rattaché au Laboratoire de Cryptogamie de l'Université de Yaoundé 1)

**Paratype** : Cameroun, lieu-dit Banla'a près de Nkongsamba-ville, altitude 878 m, à près de 420 Km au Nord-ouest de Yaoundé, 18.08.1998, collection D.C. Mossebo : "D.C.M. 55-B" (même herbier que ci-dessus).

### Commentaires.

*Nothopanus nsimalenensis* est une espèce tropicale possédant des caractères particuliers, dont certains sont uniques dans le genre *Nothopanus*. Citons ses basides clavées-cylindracées à 2 et 4 stérigmates, la présence de grosses hyphes gélatinisées et de rares pleurocystides utriformes, ainsi que son système d'hyphes dimitique avec des hyphes génératives et des hyphes squelettiques. Son principal caractère distinctif sur le plan macroscopique est la coloration de la base du chapeau vers le stipe.

Il est à relever que cette espèce pleurotoïde de la famille des Tricholomataceae (Tribu des Collybieae) est unique en son genre par ce système d'hyphes dimitique présent exclusivement chez certaines espèces de *Polyporaceae* de l'ordre des Agaricales. Cependant, la présence d'hyphes squelettiques aux côtés des hyphes génératives ne lui donne pas pour autant le caractère ligneux à dur connu par exemple des certaines espèces de *Lentinus*, la consistance de son carpophore étant au moins aussi souple que celle des pleurotes.

L'espèce dont elle se rapproche le plus, par ses caractères macroscopiques essentiellement est *Nothopanus hygrophanus* (Mont.) Sing. décrite par Pegler (1972, 1977).

Bien qu'étant décrit comme paléotropical, le genre *Nothopanus* est pour le moment peu riche en espèces. En dehors des espèces *Nothopanus lignatilis* (Pers. : Fr.) Bon et *Nothopanus porrigens* (Pers.: Fr.) Sing. décrites dans les pays d'Europe occidentale, seuls *Nothopanus hygrophanus* (Mont.) Sing. et *Nothopanus eugrammus* Mont. ont été jusqu'à ce jour décrites comme étant des espèces tropicales, mais ces deux espèces ne figurent pas dans la description faite par Hennings (1897a/b, 1902, 1907) des champignons du Cameroun.

## Discussion.

La position systématique actuelle du genre *Nothopanus* et son existence même est actuellement sujet à controverse. Singer (1986), par exemple, à la suite de Corner (1981), inclut le genre *Nothopanus* dans les *Pleurotus*, en raison de certains caractères macroscopiques et microscopiques jugés très semblables à ceux des pleurotes.

A l'inverse, Hawksworth *et al.* (1995) maintiennent l'existence du genre *Nothopanus* dans la famille des Tricholomataceae, suivant plutôt la position de Jülich (1981).

On peut rappeler que les *Pleurotus* ont pour caractères fondamentaux un système d'hyphes monomitique, avec des hyphes génératives bouclées à diamètre ± constant, des spores essentiellement cylindriques, la présence ou parfois l'absence de cheilo- et de pleurocystides et l'absence totale d'hyphes gélatinisées. Quant aux *Nothopanus*, considérés d'un point de vue général ou du point de vue particulier de *N. hygrophanus* (Mont.) Singer ex Pegler (espèce-type), ils possèdent des spores ellipsoïdes à subcylindriques, une absence totale de cheilo- et de pleurocystides, et notre *N. nsimalenensis* sp. nov. présente en plus, des hyphes génératives à deux dimensions et des hyphes squelettiques et gélatinisées.

Deux options sont possible pour classer toutes ces espèces :

- soit garder le genre *Nothopanus* avec ses caractères propres; le faible nombre d'espèces qui y sont actuellement reconnues entraînera une nécessaire révision de ses limites au fur et à mesure des nouvelles découvertes sur les espèces existantes et de la description de nouveau taxons, tel *N. nsimalenensis* sp. nov.
- soit reverser ses membres dans le genre *Pleurotus*, ce qui nécessiterait un amendement préalable des caractères généraux du genre, pour y inclure les caractères essentiels des *Nothopanus*, en général et ceux des espèces nouvellement décrites.

Chacune de ces options nécessiterait une large discussion entre experts travaillant en ce moment sur la taxonomie des deux genres en question, préalable indispensable à l'obtention d'un consensus acceptable. En particulier, l'introduction de données moléculaires plus larges que celles disponibles actuellement permettrait également d'y voir plus clair.

## REMERCIEMENTS

Nous adressons ici notre profonde gratitude au Prof. émérite Grégoire Laurent Hennebert de l'Université catholique de Louvain en Belgique pour la lecture et la révision du manuscrit ainsi que pour la mise en forme de la traduction latine. Nos remerciements vont aussi à l'endroit du Prof. Régis Courtecuisse de l'Université de Lille 2 en France pour la relecture du manuscrit et les nombreux conseils donnés pour la mise en forme de la version définitive.

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# HELICOGLOEA SPECIES COLLECTED IN TAIWAN

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## Abstract

Two new *Helicogloea* species are reported from Taiwan. *Helicogloea globosa* sp. nov. with globose basidiospores and *Helicogloea musaispora* sp. nov. with bananiform basidiospores are described, illustrated and discussed. A key to *Helicogloea* species is provided.

Key words: *Helicogloea globosa, Helicogloea musaispora,* Taiwan.

## Introduction

The genus *Helicogloea* Pat. was proposed by Patouillard & Lagerheim (1892) with a single species, *H. lagerheimii* Pat., one of the most widespread and also one of the most variable taxa of the genus. It is characterized by effused, resupinate basidiocarps with a variably thick, gelatinous subhymenium, probasidia with lateral sacs, and usually stalked metabasidia (Baker 1936). In a monograph by Baker (1946), 11 species were recognized and a key was provided. Since then, several new taxa have been proposed by various authors (Olive 1948, 1951a, 1951b, 1954, 1958a, 1958b; Kobayasi 1950; Lowy 1971; Wells, 1990). At present the genus contains 16 species. In addition, *Helicogloea sebacinoidea* L.S. Olive (Olive 1948) was erroneously based on two

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[*] Part of 180 of a series "Studies in Heterobasidiomycetes".

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different taxa (Olive 1951b), *Exidia glandulosa* and *H. sebacinoidea*. *Helicogloea terminalis* L.S. Olive (Olive 1954) has more in common with *Platygloea* s.l. and is therefore considered not to fit within the scope of *Helicogloea*. In this study we propose two new species collected in Taiwan which show the main characteristics of the genus, but differ from already known species in several features. The septal pores and spindle pole bodies of *Helicogloea* species share characteristics of the Atractiellales (Oberwinkler & Bandoni 1982; McLaughlin et al. 1988).

## Materials and Methods

Material was partly fixed in 2% glutaraldehyde and partly dried immediately after collecting. The samples were studied with a Zeiss Lab16 light microscope with phase contrast. Phloxin B or Cotton Blue were used as stains after application of 5 % KOH.

## Taxonomy

***Helicogloea globosa*** sp. nov. C.-J. Chen & Oberw. (Fig. 1)

Carposoma effusum, gelatinosum, vivens cinereum, sicco hyalinum. Hyphae 3.5-6 µm in diam., plus minusve tenuitunicatae, basaliter parce incrassatae, efibulatae. Paraphyses hyphis apparent. Probasidia clavata, 30-40(-50) x 6-8(-10) µm; metabasidia cylindracea, transverse tri-septata, 45-65 x 5-6.5 µm. Sacculi plerumque tenuitunicati, clavati, 20-25 x 6-7 µm. Basidiosporae hyalinae, tenuitunicatae, tunicis levibus, non amyloideis, globosae vel subglobosae, 8-10 x 7-9 µm, per repetitionem germinantes.
In ligno putrido crescit, Taiwan, provincia Taichung, Tashueshan, ca. 2200 m alt. Holotypus in herbario TNM in Taiwan.

Basidiocarps corticioid, ca. 35-70 µm thick, fresh whitish grey, dry hyaline to transparent. Hyphae clampless, 3.5-6 µm in diameter, slightly thick-walled in basal parts of basidiocarps. Basidia with or without short to long stalks; probasidia clavate, 30-40(-50) x 6-8(-10) µm, thin- to slightly thick-walled; metabasidia 45-65 x 5-6.5 µm, with short sterigmata, 4-spored; sacs mostly 20-25 x 6-7 µm, thin-walled, sometimes becoming enlarged when mature. Paraphyses without clamps, originating from hyphae below the sacs, growing up to the hymenial surface; usually 2-3 sacs and basidia developing from the same generative hypha. Basidiospores globose to subglobose, 8-10 x 7-9 µm, germinating by repetition, the secondary spores also globose to subglobose.

Holotypus: Taiwan, Tashueshan, ca. 2200 m alt., on wood of broad-leaved trees, in mixed montane forest with *Quercus* spp., leg. Chee-Jen Chen, June 25th, 1996, CCJ01423, deposited in the National Science Museum, Taichung, Taiwan.
Etymology: globosa - referring to globose basidiospores.

*Helicogloea globosa* is similar to *H. variabilis* K. Wells in the shape of basidiospores but differs in spore and sac measurements and in basidial morphology. *Helicogloea lagerheimii*, the commonest species in the genus, is considered to be closely related but its basidiospores are larger than those of *H. globosa*. The new species shares similar spore and sac morphology characters with *H. sphaerospora* (Möller) G.E. Baker, but lacks clamp connections. It differs from all other *Helicogloea* species with globose to oval basidiospores in spore measurements, as well as basidial and hyphal morphology.

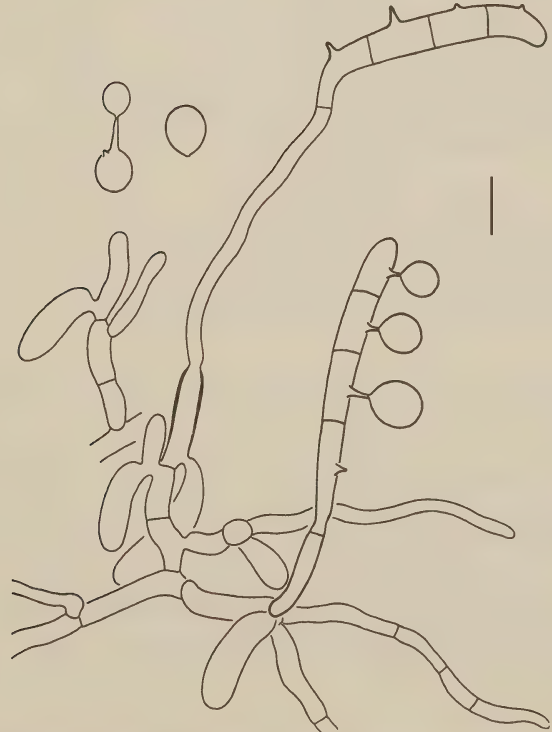

Fig. 1. *Helicogloea globosa* (from holotype). Basidia, sacs, basidiospores, and generative hyphae. Scale bar = 10 µm.

***Helicogloea musaispora*** sp. nov. C.-J. Chen & Oberw. (Fig. 2)

Carposoma effusum, gelatinosum, vivens cinereum, sicco hyalinum. Hyphae 7-12 µm in diam., plus minusve tenuitunicatae, irregulariter inflatae, efibulatae. Probasidia clavata, 13-16 x 4.5-6 µm; metabasidia cylindracea, transverse tri-septata, 55-70 x 4.5-6.5 µm; sacculi tenuitunicati, clavati, 17-26 x 8-10 µm. Basidiosporae hyalinae, tenuitunicatae, tunicis levibus, non amyloideis, naviculares an musaiforme curvatae, 18-22 x 4-5 µm, per repetitionem germinantes.
In ligno putrido crescit, Taiwan, provincia Taichung, Tashueshan, ca. 2200 m alt. Holotypus in herbario TNM in Taiwan.

Basidiocarps corticioid, mucuous-gelatinous, ca. 30-50 µm thick, fresh whitish grey, dry becoming hyaline. Hyphae without clamp connections, thin-walled to slightly thick-walled, swollen irregularly, 7-12 µm in diameter. Basidia with short stalks, probasidia thin-walled, mostly 13-16 x 4.5-6 µm; metabasidia 55-70 x 4.5-6.5 µm, 4-spored; sterigmata short, 1-2 µm in length; sacks 17-26 x 8-10 µm, thin-walled; frequently branched generative hyphae bear many sacs and probasidia. Basidiospores navicular, typically bent towards the apiculus (bananiform), 18-22 x 4-5 µm, germinating by repetition, the secondary spores similar to primary basidiospores.

Holotypus: Taiwan, Tashueshan, ca. 2200 m alt., on wood of broad-leaved trees, in mixed montane forest with *Quercus* spp., leg. Chee-Jen Chen, June 24th, 1996, CCJ 01412, deposited in the National Science Museum, Taichung, Taiwan.
Etymology: musaispora - referring to banana-like basidiospores.

*Helicogloea* species with cylindrical basidiospores are poorly investigated. The new taxon differs from all previously described *Helicogloea* species in its bananiform basidiospores and the spore measurements. Only two species, *H. longispora* Baker (1946) and *H. angustispora* L.S. Olive (1951), are considered to be closely related. *Helicogloea musaispora* differs from *H. angustispora* in hyphal width and basidial size. It is separated from *H. longispora* by considerably larger basidiospores. *Helicogloea musaispora* is the only species of the genus with bananiform basidiospores. Similar bananiform basidiospores have also been reported from *Botryobasidium bananisporum* Boidin (Boidin 1970) and *B. musaisporum* G. Langer (Langer et al. 2000).

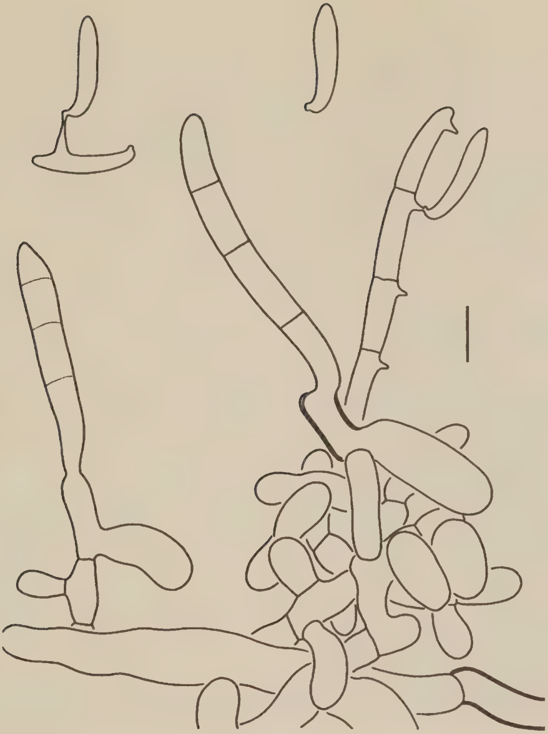

Fig. 2. *Helicogloea musaispora* (from holotype). Basidia, sacs, basidiospores, and generative hyphae. Scale bar = 10 μm.

**Key to *Helicogloea* species**

1. Clamps present ------------------------------------------------------------------------------ 2
1. Clamps absent ------------------------------------------------------------------------------- 4
2. Sacs forked --------------------------------- *H. pinicola* (Bourdot & Galzin) G.E. Baker
2. Sacs not forked ----------------------------------------------------------------------------- 3
3. Basidiospores globose, 6-8 μm in diameter ---------------------------------------------
---------------------------------------------------- *H. sphaerospora* (Möller) G.E. Baker
3. Basidiospores ovoid, 8-12 x 5-8 μm-------------- *H. graminicola* (Bres.) G.E. Baker
4. Basidiospores septate ---------------------------------------------------------------------- 5
4. Basidiospores without septa ---------------------------------------------------------------- 8
5. Basidiospores 1-3 septate ------------------------------------------------------------------ 6

5. Basidiospores 0-1 septate ---------------------------------------- 7
6. Basidiospores 15.5-18 x 7-8 µm ---------------------------- *H. inconspicua* G.E. Baker
6. Basidiospores 12-15 x 9-10 µm ---------------------------- *H. incrustans* Kobayasi
7. Basidiospores ellipsoid, 14-24 x 5.5-9 µm, sacs 28-39 x 7.0-11 µm, metabasidia 65-72 x 5.5-9 µm ---------------------------- *H. intermedia* (Linder) G.E. Baker
7. Basidiospores cylindrical, 16.5-32 x 5-8.5 µm, sacs 25-50 x 10-14 µm, metabasidia 70-122 x 6.5-8 µm, parasitic in *Exidia glandulosa*---------*H. parasitica* L.S. Olive
8. Basidiocarps usually a thin mucous-gelatinous film ---------------------------- 9
8. Basidiocarps a well-developed, crumpled to convoluted cushion, pulvinate, up to several mm thick; basidiospores 15-17 x 6-7.5 µm, sacs 25-45 x 8.5-16 µm*H. caroliniana* (Coker) G.E. Baker
9. Basidiocarps becoming golden-orange when dry ---------------*H. aurea* G.E. Baker
9. Basidiocarps lacking strong pigments when dry---------------------------- 10
10. Hyphae irregularly coiled ---------------------------- 11
10. Hyphae not coiled ---------------------------- 12
11. Basidiospores ovoid, 12-15 x 7.5-9 µm-----------------------*H. contorta* G.E. Baker
11. Basidiospores unequal to crescent, 24-31 x 9-11 µm ------------- *H. indica* Boedijn
12. Basidia 1-2 septate ----------------------------*H. irregularis* L.S. Olive
12. Basidia 3 septate---------------------------- 13
13. Basidiospores globose, subglobose to ovoid ---------------------------- 14
13. Basidiospores fusiform to cylindrical ---------------------------- 16
14. Basidiospores smaller than 10 µm, metabasidia 45-65 x 5-6.5 µm, sacs 20-25 x 6-7 µm ----------------------------***H. globosa* sp. nov.**
14. Basidiospores larger than 10 µm ---------------------------- 15
15. Basidiospores 10.5-13 x 7.5-10.5 µm, metabasidia 48-83 x 6.5-10.5 µm, sacs 6-17.5 x 4.5-12.5 µm ---------------------------- *H. variabilis* K. Wells
15. Basidiospores 13-18 x 6.5-8 µm, metabasidia 69-100 x 5-7.5 µm, sacs 18-38 x 5.5-10 µm ----------------------------*H. lagerheimii* Pat.
16. Sacs smaller than 8 µm, 14.5-32 x 5-6.5 µm, metabasidia 36-66 x 3-4 µm, basidiospores 9-26.5 x 2.5-4.5 µm------------------------ *H. angustispora* L.S. Olive
16. Sacs broader than 8 µm---------------------------- 16
17. Basidiospores 13-15 x 4.5-7.5 µm, metabasidia 65-70 x 5-8 µm, sacs 45-50 x 9-11 µm ----------------------------*H. longispora* G.E. Baker
17. Basidiospores 18-22 x 4-5 µm, metabasidia 55-70 x 4.5-6.5 µm, sacs 17-26 x 8-10 µm ----------------------------***H. musaispora* sp. nov.**

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# PHELLINUS TABAQUILIO SP. NOV FROM CÓRDOBA MOUNTAINS, CENTRAL ARGENTINA

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## Abstract

*Phellinus tabaquilio* is described as new. It was found on living *Polylepis australis*, Rosaceae, in Córdoba mountains, central Argentina. It belongs to the *Phellinus robustus* complex (Hymenchaetaceae, Aphyllophorales) being distinguished by its macromorphology, the way it grows and the substrate.

**Key words**: *Phellinus robustus* complex, Aphyllophorales, *Polylepis australis*.

## Introduction

Some poroid Hymenochaetaceae (Aphyllophorales) have been reported for lower vegetation belts in the mountains of central Argentina (Spegazzini 1926; Urcelay & Rajchenberg 1999; Urcelay et al. 1999). Nevertheless, no species has been collected at higher altitudes where "tabaquillo" (*Polylepis australis*, Rosaceae) forms monospecific forest patches above 1800 masl (Cabido & Acosta 1985). Several visits to the aforementioned forests have revealed some new and interesting taxa, one of which is described here.

## Materials and Methods

The study of the material followed the usual methodology proposed for these type of fungi (Ryvarden & Johansen 1980; Gilbertson & Ryvarden 1986). Microscopic structures were observed using an optic microscope KYOWA (x 1000). The collections were deposited in the herbarium of the "Museo Botánico de la Facultad de Ciencias Exactas, Físicas y Naturales de la Universidad Nacional de Córdoba" (CORD).

## Results

***Phellinus tabaquilio*** Urcelay, Robledo & Rajchenb. sp. nov. Figs. 1-7

*Basidiocarpo perenni, resupinato ad effuso-reflexo, solitario, usque ad 10 x 20 x 5 cm, superficie pilei aureobrunnea concentrice sulcata deinde nigra rimosa. Contexto ligneo, pauce crassitudine, aureobrunneo vivido. Hymenophoro tubulari, eodem colore quam contexto. Poris aureocastaneis, circularibus, 4-6 per mm. Basidiis ample claviformibus ad ellipsoideum, tetrasporis, 12-14,5 x 8-10 µm. Cystidiolis tenuis, ventricosis, 16-29 x 3-5 µm. Setis nullis. Basidiosporis subglobosis, crassitunicatis, hyalinis, apiculatis, 6-7,5 x (4,5-) 4,8-6,5 µm, dextrinoideis, cyanophilis. Systema hypharun cum generativis tenuitunicatis, 1,5-2,5 µm diam. et sceletis brunneis, leviter crassetunicatis, 1,5-3,5 µm diam.*

***Holotypus****. Argentina, Córdoba, Los Gigantes, leg. Robledo et Urcelay n° 194, 31.I.2000 in Herb. CORD conservatus est; isotypus BAFC.*

**Basidiocarp** perennial, the first layer(s) resupinate to effuse, later layers growing one below the other but forward and expanding in relation with the previous one, the basidiocarp becoming effuse-reflexed, pendent, and finally forming a thick triquetrous to ungulate basidiocarp, up to 10 x 20 x 5 cm. Pilear surface strongly sulcate, furrows narrow, irregular and strongly tuberculate in some portions, formed by each layer's margin growing upwards, hard, corky, indurating and becoming rimose close to the substrate; dark brown, but lighter towards the margin. Margin sterile and blunt. Pore surface golden brown, glancing, pores 4-6/mm, circular, with smooth, round dissepiments. Context fibrous, bright golden brown, poorly developed but present between each stratum. Tubes concolorous, up to 4 mm long.

**Hyphal system** dimitic. Contextual skeletal hyphae brown, up to 4 µm diam. Generative hyphae simple septate, thin-walled, hyaline, 1,5-2,5 µm diam.; tramal skeletal hyphae thick-walled, brown, 1,5-3,5 µm diam.

**Setae and setal hyphae** absent.

**Cystidioles** ventricose, hyaline, thin-walled, with inflated base and elongated thin apex, 16-29 x 3-5 µm, apical portion 1,5-2,5 µm diam.

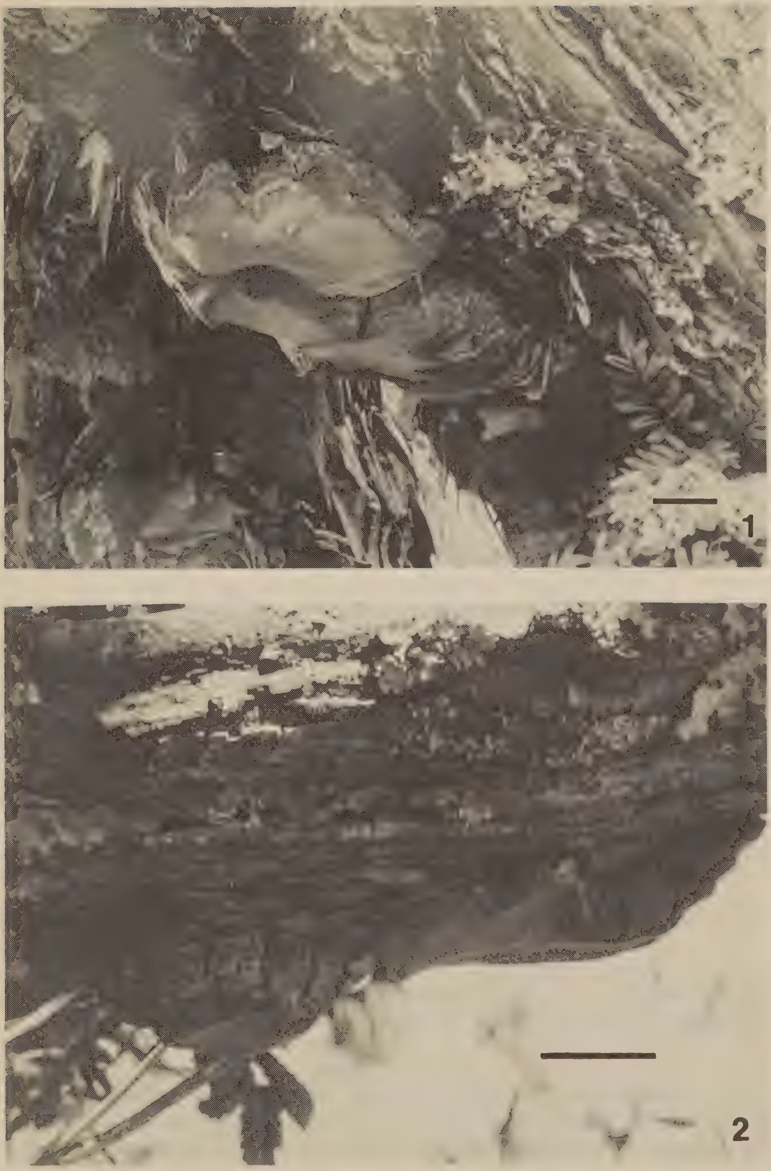
**Basidia** broadly clavate to ellipsoid, 12-14,5 x 8-10 µm, with 4 sterigmata.

**Basidiospores** subglobose, 6-7,5 x (4,5-) 4,8-6,5 µm, with thickened, hyaline walls, dextrinoid and cyanophilous.

**Associated wood-root** white.

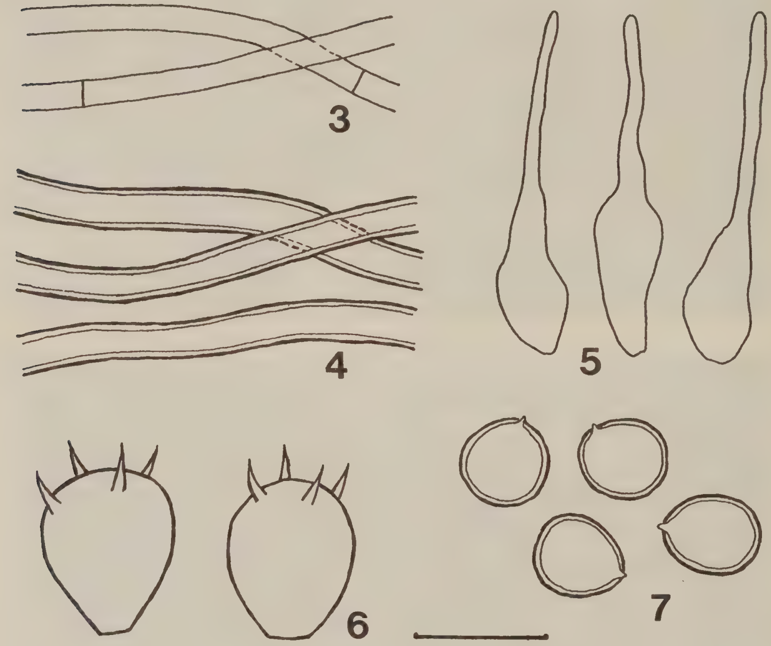
**Substrate**: on living 'tabaquillo' or 'queñoa' (*Polylepis australis*, Rosaceae), a tree that forms isolated forest at high altitudes (1900-3000 m.a.s.l.) in the 'Bosques Montanos' District of the subtropical Yunga Forest of Argentina (Cabrera 1971) and at 1300-2600 m.a.s.l. high in the 'Chaco Serrano' District of Central Argentinean mountains (Luti et al., 1979).

**Material studied**: ARGENTINA, Córdoba, Depto. Punilla, Los Gigantes, Valle de los lisos, leg. C. Urcelay 69, 20.Feb.99 (CORD). Ibid., leg. G.L. Robledo & C. Urcelay 189, 193, 194 and 195, 31.Jan.00 (CORD). Depto. San Alberto, Pampa de Achala between La Pampilla and El Cóndor, leg. V. Ceaglio, G.L. Robledo & C. Urcelay 173, 174, 176, 179 and 180, 28.Nov.99 (CORD). Ibid., leg. G.L. Robledo 10, 12.Mar.00 (CORD).



Figs.1-2. *Phellinus tabaquilio*, macroscopical features: 1, resupinate to effuse basidiocarp (Robledo n° 10). 2, triquetrous to ungulate basidiocarp with rimose pileus (Holotypus). Bars= 2,5cm.

**Remarks**: The new species clearly belongs in the *Phellinus robustus* (P. Karst.) Bourdot & Galzin complex of species, being distinguished by its macromorphology, how it grows and the substrate. In subtropical Argentina the only other pileate species belonging in this group is *Phellinus apiahynus* (Speg.) Rajchenb. & Wright, which differs in smaller pores (7-8/mm), indistinctly stratified tubes and smaller spores (5-6 x 4.5-5 μm) (Wright & Blumenfeld 1984 as *Phellinus elegans* J.E. Wright & Blumenf., Rajchenberg & Wright 1987).



Figs. 3-7, *Phellinus tabaquilio*, microscopical features: 3, generative tramal hyphae. 4, skeletal tramal hyphae. 5, cistidioles. 6, basidia. 7, basidiospores. Bar= 10μm

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# NEW SPECIES OF PARMELIACEAE (LICHENIZED ASCOMYCOTINA) FROM CHINA

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ABSTRACT: Four new lichen species are described from China: *Bulbothrix yunnana* S. L. Wang, J. B. Chen & Elix, *Hypotrachyna asiatica* S. L. Wang, J. B. Chen & Elix, *H. ciliata* S. L. Wang, J. B. Chen & Elix and *Parmelina gyrophorica* Elix, S. L. Wang & J. B. Chen.

KEY WORDS: lichen; *Bulbothrix; Hypotrachyna; Parmelina*

## Introduction

As a result of our continuing study of the lichen family Parmeliaceae in China (Wang, Chen & Elix 2000), we are describing a further four new species. Throughout the present work chemical constituents were identified by thin layer chromatography (Culberson 1972; Culberson *et al.* 1981; Culberson & Johnson 1982; Elix & Ernst-Russell 1993), high performance liquid chromatography (Elix *et al.* 1997; Feige *et al.* 1993) and comparison with authentic samples.

**Bulbothrix yunnana** S. L. Wang, J. B. Chen & Elix, sp. nov. Fig. 1

Thallus ut in *Bulbothrix suffixa* sed ciliis contractis, parum tumidis, lobulatis eciliatis, et acidum caperaticum et acidum aliphaticum incognitum continente differt.

*Type*: CHINA. Yunnan: Zhongdian County, 3700 m, on bark of *Acer*, *X. Y. Wang, X. Xiao, J. J. Su 5669*, 14 Aug. 1981 (holo: HMAS-L; iso: CANB).

*Thallus* corticolous, foliose, coriaceous, adanate, 3-5 cm wide. *Lobes* separate, sublinear to subirregular, subirregularly to subdichotomously branched, apically truncate, 1-3 mm wide; the margin with a narrow black rim, ciliate, cilia tapered or weakly inflated at the base, ±apically branched, irregularly dispersed but very dense in the lobe axils, 0.8-1.2 mm long. *Upper surface* pale whitish grey to greenish mineral grey, flat, shiny at first, distinctly white-maculate on older lobes, lacking soredia and isidia but with dense laminal lobules; lobules procumbent, spathuliform to ovoid or helmet shaped, eciliate. *Medulla* white. *Lower surface* black; rhizines dense, black, squarrosely to intricately branched and agglutinated, ±originating from bulbae, to 1.5 mm long. *Apothecia* rare, sessile, 1-3 mm wide; disc concave, dark brown, thalline exciple ecoronate; ascospores ellipsoidal, 10-12.5 x 4-5µm. *Pycnidia* not seen.

*Chemistry*. Cortex: K+ yellow; medulla K-, C-, KC-, P-; containing atranorin (major), chloroatranorin (minor), caperatic acid (major) and an unidentified fatty acid (minor).

This species is characterized by the narrow ciliate lobes with truncate apices, the presence of dorsiventral, oblong or spathuliform to ovoid lobules and dense, squarrose to agglutinated rhizines. The cilia are tapered but are not or are only weakly inflated. Morphologically this new species most closely resembles *Bulbothrix suffixa* (Stirt.) Hale (Hale 1976) , but the latter species can be distinguished by the presence of distinctly inflated bulbate cilia, lobulae which are marginally bulbate-ciliate and the presence of medullary gyrophoric acid. At present *B. yunnana* is known from several high elevation locations in Yunnan Province, southern China whereas *B. suffixa* is more widely distributed in tropical America and South Africa (Hale 1976).

*Paratype Specimens Examined*

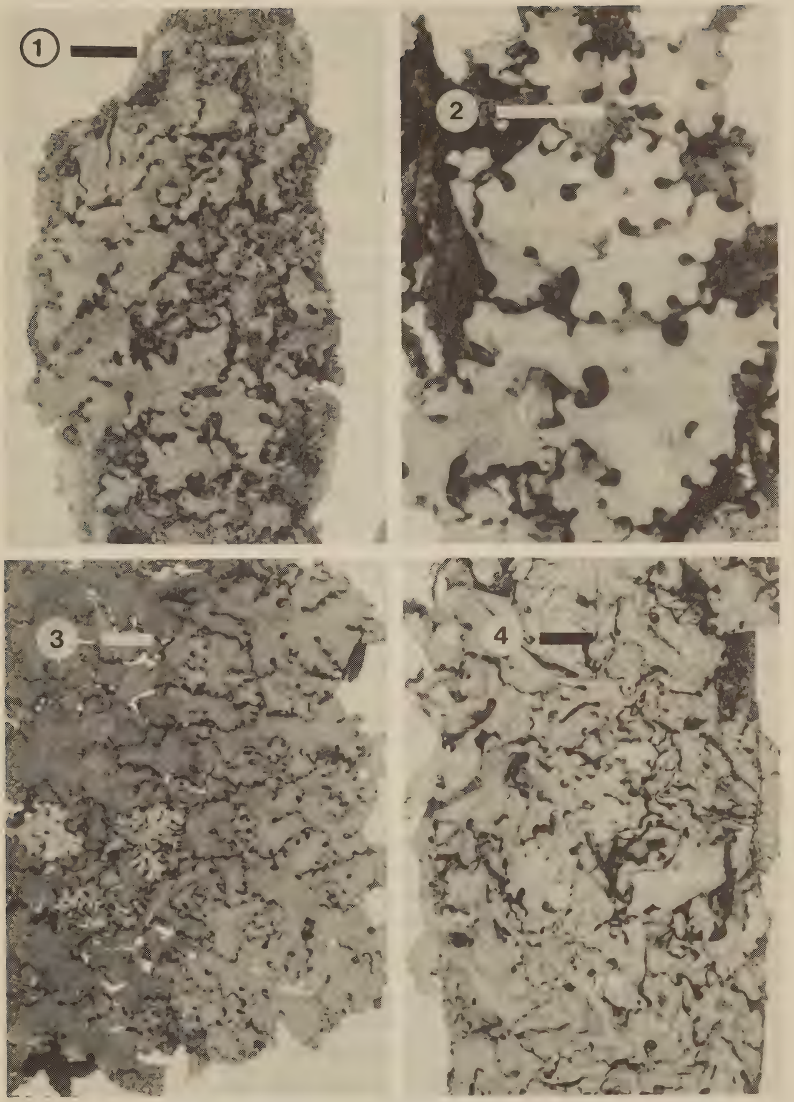
CHINA. Yunnan, Gongshan County, 1400 m, on bark, *J. J. Su 3162*, 22 Aug. 1982 (HMAS-L); Gongshan County, 1650 m, on bark, *X. Y. Wang, X. Xiao, J. J. Su 3434*, 24 Aug. 1981 (HMAS-L); Zhongdian County, 3700 m, *X. Y. Wang, X. Xiao, J. J. Su 5381*, 14 Aug. 1981 (HMAS-L); Zhongdian County, Mt Tianbao, 3700 m, on bark of fir, *X. Y. Wang, X. Xiao & J. J. Su 5753, 5756*, 19 Aug. 1981 (HMAS-L).

**Hypotrachyna asiatica** S. L. Wang, J. B. Chen & Elix, sp. nov. Fig. 2

Thallus ut in *Hypotrachyna majoris* sed esorediatis differt.

*Type*: CHINA. Yunnan, Mt. Gongshan, 2200 m, on bark, *J. J. Su 2761*, 6 Aug. 1982 (holo: HMAS-L; iso: CANB).

*Thallus* corticolous, foliose, adnate, 2-3 cm wide. *Lobes* imbricate, sublinear to subirregular, subdichotomously to irregularly branched, 1.5-2.0 mm wide, apically truncate; the margin ciliate, cilia rarely simple but commonly furcate, dense, 0.2-0.5 mm long. *Upper surface* pale grey to greenish grey, flat, shiny, weakly maculate, rugulose, without soredia, pustules, isidia or



Figures 1-4. New species of Parmeliaceae: 1, *Bulbothrix yunnana* (holotype in HMAS-L); 2, *Hypotrachyna asiatica* (holotype in HMAS-L); 3, *H. ciliata* (holotype in HMAS-L); 4, *Parmelina gyrophorica* (holotype in HMAS). Scale bar = 5 mm.

lobules. Medulla off-white. *Lower surface* black; densely rhizinate, rhizines richly dichotomously branched, often projecting beyond the lobe margins. One immature apothecium observed but no spores seen. *Pycnidia* common, immersed, puctiform. *Conidia* weakly bifusiform, 4-6 x 1 µm.

*Chemistry*: Cortex K+ yellow; medulla K+ red, C-, KC-, P+ yellow-orange; containing atranorin (major), salazinic acid (major), zeorin (major), consalazinic acid (minor), galbinic acid (trace).

This species is characterized by the abscence of vegetative propagules, the richly branched, dichotomous rhizines and the presence of salazinic acid, consalazinic acid and zeorin in the medulla. Chemically *H. asiatica* is identical with *H. majoris* (Vain.) Hale (Hale & Kurokawa 1964), but the latter can be distinguished by the presence of soredia and the sparsely branched, dichotomous rhizines. This new species could be confused with *Hypotrachyna coorgiana* Patwardhan & Prabhu from India and Thailand, since both lack vegetative propagules and contain medullary salazinic acid (Patwardhan & Prabhu 1977). However, *H. coorgiana* differs in having broader, involute lobes (2-3 mm *cf.* 1.5-2 mm wide) and sparse rhizines, whereas *H. asiatica* has narrower, flat lobes and very dense rhizines. At present this new species is only known from the type locality.

**Hypotrachyna ciliata** S. L. Wang, J. B. Chen & Elix, sp. nov. Fig. 3

Thallus ut in *Hypotrachyna scytophylla* sed lobis veteribus lobulatis differt.

*Type*: CHINA. Yunnan, Mt. Yulongshan, 3000 m, on rocks, *J. J. Su 5013*, 4 Aug. 1981 (holo: HMAS-L; iso: CANB).

*Thallus* corticolous, foliose, adnate, 2-3 cm wide. *Lobes* imbricate, sublinear to subirregular, subdichotomously to irregularly branched, 2-4 mm wide, apically truncate; the margin ciliate, cilia simple or furcate, 0.2-0.7 mm long, mainly in the lobe axils. *Upper surface* pale grey to mid-grey, flat, shiny, with a narrow black margin, lacking soredia and isidia but older lobes becoming densely lobulate; lobules laminal and marginal, adnate or imbricate, sublinear to subirregular, 0.5-1.0 mm wide. *Medulla* white. *Lower surface* black, moderately rhizinate, with a narrow, dark brown, naked marginal zone; rhizines moderately dichotomously branched or rarely simple, 0.8-1.0 mm long. *Apothecia* not seen. *Pycnidia* common, immersed, puctiform. *Conidia* weakly bifusiform, 4-6 x 1 µm.

*Chemistry*: Cortex K+ yellow; medulla K-, C+ rose, KC+ rose, P-; containing gyrophoric acid (major), atranorin (minor), chloroatranorin (minor), lecanoric acid (trace).

Morphologically this new species resembles *Hypotrachyna scytophylla* (Kurok.) Hale (Hale & Kurokawa 1964) as both have relatively broad, sublinear to subirregular lobes with ciliate margins, lack soredia and isidia, have sparely branched dichotomous rhizines and contain medullary gyrophoric acid. However, *H. ciliata* can readily be distinguished by the presence of marginal and laminal lobules on the upper surface. At present this species is only known from the type locality in Yunnan Province, southern China.

**Parmelina gyrophorica** Elix, S. L. Wang & J. B. Chen, sp. nov. Fig. 4

Thallus ut in *Parmelina pseudorelicina* sed lobis latioribus et acidum gyrophoricum continente differt.

*Type*: CHINA. Yunnan: Gongshan County, Cikaipuladadui, 3250 m, on dead wood, *J. J. Su 2539*, 25 July 1982 (holo: HMAS-L; iso: CANB).

*Thallus* corticolous, foliose, adanate, 3-5 cm wide. *Lobes* imbricate, subirregular, irregularly branched, apically subrotund, 2-5 mm wide; the margin ciliate, cilia sparse, simple, irregularly dispersed, often confined to the lobes axils, 0.1-0.5 mm long. *Upper surface* pale grey to grey-green, flat, shiny, emaculate, smooth or weakly foveolate at first becoming rugulose, lacking soredia and isidia, but with sparse laminal lobules. *Medulla* white. *Lower surface* black, with a very narrow, dark brown, naked marginal zone; rhizines moderate, simple, black, 0.3-1.0 mm long. *Apothecia* rare, only immature discs seen. *Pycnidia* common, laminal, immersed, punctiform. *Conidia* bacilliform, 5-6 x 1 µm.

*Chemistry*. Cortex: K+ yellow; medulla K-, C+ rose, KC+ red, P-; containing atranorin (major), chloroatranorin (minor), gyrophoric acid (major), lecanoric acid (minor/trace), orsellinic acid (trace), orcinol (trace).

This species is characterized by thalli with emaculate, sparsely ciliate lobes, the lack of soredia and isidia, the presence of simple rhizines and medullary gyrophoric acid. Morphologically this new species resembles *Parmelina pseudorelicina* (Jatta) Kantvilas & Elix and *P. quercina* (Willd.) Hale (Elix 1994). However, both of the latter species can be distinguished by the presence of significantly narrower lobes (0.5-3.0 mm *cf.* 2.0-5.0 mm wide), and the presence of medullary lecanoric acid rather than gyrophoric acid. In addition, *P. quercina* has lobes which are distinctly white-maculate on the upper surface. At present *P. gyrophorica* is known only from the type locality in Yunnan Province, southern China, whereas *P. pseudorelicina* is common in Australia and New Zealand, and *P. quercina* is a cosmopolitan species found in temperate regions throughout the world.

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# MELIOLACEAE OF KERALA, INDIA - VII

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## Abstract

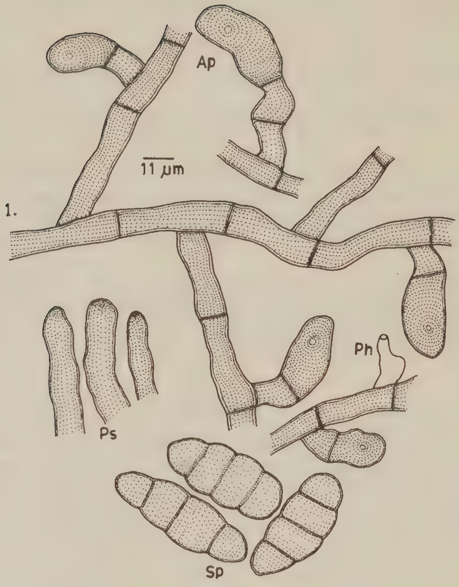
This paper gives an account of four Meliolaceae from Kerala, India. Of these, *Irenopsis vaccinii, Meliola cholakadensis* and *M. mannavanensis* are new species, while *Meliola oldenlandiae* Hansf. & Stev. is reported herein for the first time from India.

**Key Words:** Irenopsis, Meliola.

**1.** ***Irenopsis vaccinii*** sp. nov. (Fig. 1)

Coloniae amphigenae, densae, dispersae, ad 2 mm diam., raro confluentes. Hyphae subrectae vel anfractuae, plerumque alternate acuteque ramosae, laxe vel raro dense reticulatae, cellulis 12-33 µ 9-11 µm. Appressoria alternata, antrorsa vel patentia, recta vel curvula, 30-50 µm longa; cellulae basalis plerumpque unicellulae, raro uniseptatae, rectae, flexuosae vel curvulae, 10-23 µm longae; cellulae apicalis ovatae, oblongae vel cylindraceae, rectae vel curvulae, integrae vel sinuatae, 20-28 × 12-20 µm. Phialides paucae, appressoriis intermixtae, alternatae, ampulliformes, 20-25 × 7-10 µm. Perithecia dispersa, verrucosa, ad 200 µm diam.; setae peritheciales 10-15, rectae vel modice curvulae, septatae, glabrae, obtusae ad apicem, ad 178 µm longae; ascosporae ellipsoideae, rectae vel modice curvulae, 3-septatae, constrictae, 50-55 × 20-23 µm.

Colonies amphigenous, dense, scattered, up to 2 mm in diam., rarely confluent. Hyphae substraight to crooked, branching mostly alternate at acute angles, loosely to rarely densely reticulate, cells 12-33 × 9-11 µm. Appressoria alternate, antrorse to spreading, straight to curved, 30-50 µm long; stalk cells cylindrical, mostly unicellular, rarely 1-septate, straight, flexuous to curved, 10-23 µm long; head cells ovate, oblong to cylindrical, straight to curved, entire to sinuate, 20-28 × 12-20 µm. Phialides few, mixed with appressoria, alternate, ampulliform, 20-25 × 7-10 µm. Perithecia scattered, verrucose, up to 200 µm in diam.; perithecial setae 10-15, straight to slightly curved, septate, smooth walled, obtuse at the apex, up to 178 µm long; ascospores ellipsoidal, straight to slightly curved, 3-septate, constricted at the septa, 50-55 × 20-23 µm.



1. *Irenopsis vaccinii* sp. nov. Ps = Perithecial setae, Ph = Phialides, Ap = Appressoria, Sp = Ascospores.

***Holotype:*** On leaves of *Vaccinium leschenaultii* Wight (Vacciniaceae), Rajamala, Idukki, Kerala, India, September 11, 1998, C.K. Biju ILLS 54759. ***Isotype:*** TBGT 230.

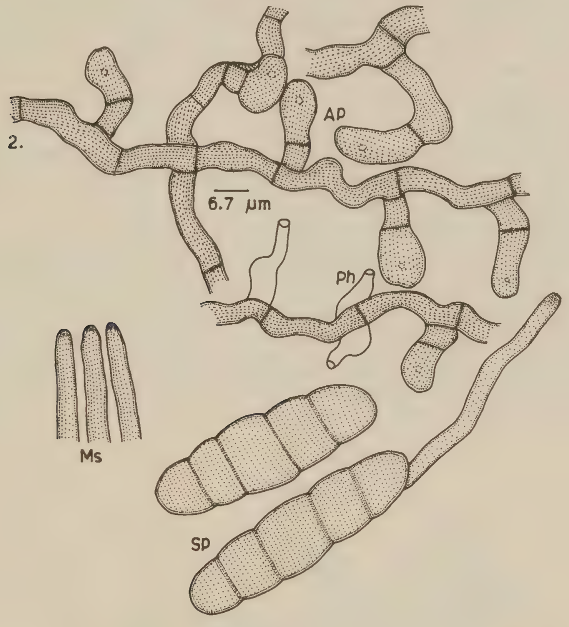
*Irenopsis sinsuiensis* Yaman is known on *Vaccinium caudatifolium* Hayata from Taiwan (Yamamoto, 1940); however, the new species differs from it in having smaller, 3-septate ascospores.

**2.** ***Meliola cholakadensis*** sp. nov. (Fig. 2)

ETYMOLOGY: Cholakad in Malayalam, Shola forest.

Coloniae hypophyllae, tenues vel densae, ad 5 mm diam., confluentes. Hyphae undulatae vel anfractuae, opposite vel irregulariter laxe ramosae, laxe vel arte reticulatae, cellulis 30-38 × 5-8 µm. Appressoria alternata, recta vel curvula, patentia, 20-38 µm longa; cellulae basalis cylindraceae vel cuneatae, rectae vel curvulae, unicellulae, raro uniseptatae, 5-18 µm longae; cellulae apicalis globosae, ovatae, hamatae, integrae, 15-23 × 12-23 µm. Phialides appressoriis intermixtae, alternatae vel oppositae, ampulliformes, 15-38 × 7-10 µm. Setae myceliales numerosae, dispersae vel plerumque circa perithecia aggregatae, simplices, rectae vel leniter curvulae, acutae vel obtusae ad apicem, ad 460 µm longae. Perithecia dispersa, verrucosa, ad 250 µm diam.; ascosporae oblongae vel subellipsoideae, 4-septatae, constrictae, cellulae centralis magniorae, 45-53 × 17-20 µm.

Colonies hypophyllous, thin to dense, up to 5 mm in diam., confluent. Hyphae undulate to crooked, branching opposite to irregular at wide angles, loosely to closely reticulate, cells 30-38 × 5-8 µm. Appressoria alternate, straight to curved, spreading, 20-38 µm long; stalk cells cylindrical to cuneate, straight to curved, 5-18 µm long; head cells globose, ovate, hamate, entire, 15-23 × 12-23 µm. Phialides mixed with appressoria, more in the branches where appressoria are lesser in number, alternate to opposite, ampulliform, 15-38 × 7-10 µm. Mycelial setae numerous, scattered to mostly grouped around perithecia, simple, straight to slightly curved, acute to obtuse at the tip, up to 460 µm long. Perithecia scattered, verrucose, up to 250 µm in diam.; ascospores oblong to subellipsoidal, 4-septate, constricted, central cell slightly larger, 45-53 × 17-20 µm.



2. *Meliola cholakadensis* sp. nov. Ms = Mycelial setae, Ph = Phialides, Ap = Appressoria, Sp = Ascospores.

***Holotype:*** On leaves of an unidentified Lauraceae, Rajamala, Idukki, Kerala, India, September 8, 1998, C.K. Biju ILLS 54760. ***Isotype:*** TBGT 231.

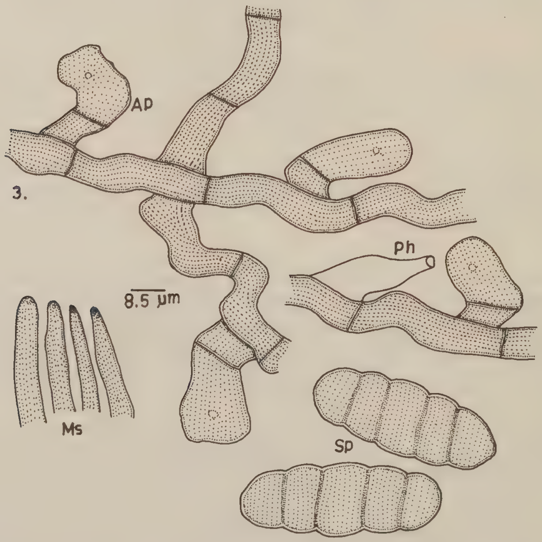
The new species is similar to *Meliola sempeiensis* Yamam. Known on *Nothaphobe kenoshii* (Hayata) Hayata from Taiwan (Yamamoto, 1941); however, differs from it in having longer and variously curved appressoria, straight mycelial setae and smaller ascospores (Hansford, 1961; Hosagoudar, 1996; Hosagoudar *et al.*, 1997).

### 3. *Meliola mannavanensis* sp. nov. (Fig. 3)

ETYMOLOGY: After the type locality, Mannavan.

Coloniae hypophyllae, dispersae, subdensae vel densae, ad 5 mm diam., raro confluentes. Hyphae flexuosae vel anfractuae, opposite vel irregulariter acuteque ramosae, laxe vel arte reticulatae, cellulis 22-35 × 10-13 µm. Appressoria dispersa, alternata, patentia vel irregulariter curvula, 15-33 µm longa; cellulae basalis cylindraceae vel cuneatae, 5-13 µm longae; cellulae apicalis ovatae, globosae, oblongae, irregularae, curvulae, integrae vel angularae, 10-20 × 12-18 µm. Phialides numerosae, appressoriis intermixtae, alternatae vel oppositae, ampulliformes, 15-35 × 7-10 µm. Setae myceliales dispersae vel circa perithecia aggregatae, simplices, hamatae, arcuatae vel irregulariter curvulae, obtusae vel acutae ad apicem, ad 450 µm longae. Perithecia dispersa vel aggregata, verrucosa, ad 225 µm diam.; ascosporae ellipsoideae, 4-septatae, constrictae, cellulae centralis magniorae, 50-53 × 15-18 µm.

Colonies hypophyllous, scattered, subdense to dense, up to 5 mm in diam., rarely confluent. Hyphae flexuous to crooked, branching opposite to irregular at acute angles, loosely to closely reticulate, cells 22-35 × 10-13 µm. Appressoria scattered, alternate, antrorse, spreading to irregularly curved, 15-33 µm long; stalk cells cylindrical to cuneate, 5-13 µm long; head cells ovate, globose, variously curved, entire to angular, 10-20 × 12-18 µm. Phialides many, mixed with appressoria, alternate to opposite, ampulliform, 15-35 × 7-10 µm. Mycelial setae scattered to mostly grouped around perithecia, simple, hamate,



3. *Meliola mannavanensis* sp. nov. Ms = Mycelial setae, Ph = Phialides, Ap = Appressoria, Sp = Ascospores.

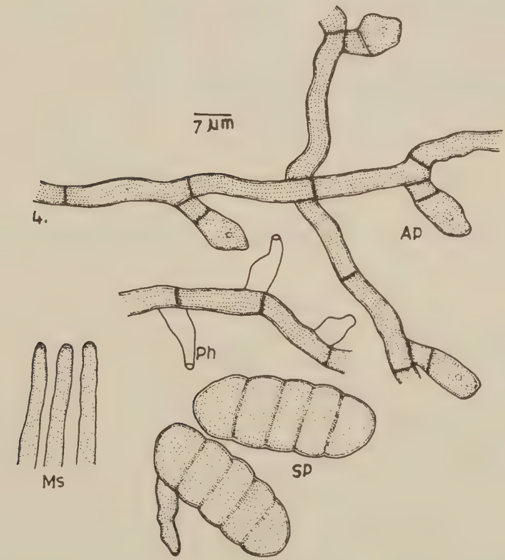
arcuate to irregularly curved, obtuse at the apex, up to 450 µm long. Perithecia scattered to grouped, verrucose, up to 225 µm in diam.; ascospores ellipsoidal, 4-septate, constricted at the septa, central cell slightly larger, 50-53× 15-18 µm.

***Holotype:*** On leaves of *Litsea* sp. (Lauraceae), Mannavan Shola, Idukki, Kerala, India, September 23, 1998, C.K. Biju ILLS 54761. ***Isotype***: TBGT 232.

The new species is similar to *Meliola drepanochaeta* Sydow known on *Persea cordata* (Vell.) Mez = *P. caerulea* (Ruiz & Pav.) Mez from Costa Rica (Hansford, 1961); however, it differs in having shorter appressoria, entire to angular head cells of appressoria and smaller ascospores.

4. ***Meliola oldenlandiae*** Hansf. & F. Stev. in Hansf., J. Linn. Soc. London 5:279, 1973; Hansf., Sydowia Beih. 2:596, 1961. (Fig. 4)

Colonies amphigenous, thin to dense, up to 2 mm in diam. Hyphae substraight to flexuous, branching opposite to irregular at acute to wide angles, loosely to closely reticulate, cells 27-38 × 5-8 µm. Appressoria alternate, antrorse, 17-25 µm long; stalk cells cylindrical to cuneate, 5-10 µm long; head cells ovate, globose, entire, angular, attenuated and rounded at the apex, 12-15 × 10-13 µm. Phialides borne on a separate mycelial branch, alternate to opposite, ampulliform, 12-18 × 6-8 µm. Mycelial setae numerous, scattered to grouped around perithecia, straight to curved, not uncinate, obtuse at the apex, up to 420 µm long. Perithecia scattered to grouped, verrucose, up to 175 µm in diam.;



4. *Meliola oldenlandiae* Hansf. & Stev. Ms = Mycelial setae, Ph = Phialides, Ap = Appressoria, Sp = Ascospores.

ascospores obovoidal, 4-septate, slightly constricted at the septa, 35-38 × 12-15 µm.

On leaves of *Hedyotis* sp. (Rubiaceae), Mannavan Shola, Idukki, Kerala, India, October 23, 1998, C.K. Biju ILLS. 54763 TBGT 233.

This species was known only from the type collection on *Oldenlandia decumbens* (Hochst.) Hiern from Uganda (Hansford, 1961) and this collection represents a new host record and new record to India.

## ACKNOWLEDGEMENTS

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# POTENTIAL USE OF RESTRICTION ANALYSIS OF PCR-AMPLIFIED DNA FRAGMENTS IN CONSTRUCTION OF MOLECULAR DATA-BASED IDENTIFICATION KEYS OF LICHENS

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## Abstract

Identification of lichen species solely on the basis of morphological and anatomical features of samples is often difficult. Molecular biology techniques provide many opportunities in taxonomy, but these techniques are usually used for determination of phylogenetic relationships at different levels rather than for identification of particular specimens. Here we asked whether restriction analysis of PCR-amplified DNA fragments can be used as a diagnostic test for identification of particular taxa. PCR amplification and rDNA restriction fragment length polymorphism (RFLP) were used to examine variability among species of the genus *Melanelia*. Restriction patterns were found to be characteristic for particular species, though in some cases more than one pattern occurred in one species. Therefore, we propose that restriction patterns of PCR-amplified DNA fragments may be used for identification of lichens, especially for taxa difficult to distinguish on the basis of morphological and anatomical features. Possible usefulness of identification keys based on DNA restriction patterns is discussed.

**Key words**: Lichens, molecular taxonomy, polymerase chain reaction (PCR), rDNA amplification, restriction analysis

## Introduction

Molecular biology techniques provide many opportunities for taxonomical studies, including systematics of lichenized fungi (for a review see Bridge & Hawksworth, 1998). Nucleotide sequence analysis of certain genome fragments allows detection of variability between organisms at the level of a single base pair, and thus it can be used to determine relationships between different taxa. Using the polymerase chain reaction, PCR (for a review of this method see Karcher, 1995), it is possible to amplify a desired specific DNA fragment starting from a very small amount of impure material (e.g. cell extract) as a template. This technique has been proposed as a method for detection of the presence of the particular species of fungi by using specific primers, complementary to certain fragments of the genome of the individuals from the given species, but not complementary to DNA of other organisms (see for example: Fulton & Brown, 1997; Johanson *et al.*, 1998). In such cases, the specific PCR product is detected only when fragments of the genome of the individuals belonging to the given species are present in the analyzed sample.

Apart from finding relationships between taxa and determination whether organisms belonging to a particular species are present in the investigated sample of material, another taxonomical problem exists. This problem is the proper identification of investigated collections. Many genera of lichens contain species that are morphologically and anatomically similar. Thus, proper and unambiguous identification of investigated samples is sometimes difficult. Molecular biology techniques may potentially provide opportunities for clear identification of a particular specimen. However, DNA sequencing is time consuming and too expensive if one wanted to use comparison of nucleotide sequences for identification of many collections. Identification of DNA from one species using primers specific exclusively for regions of the genome of this species is very useful for detection of the presence of samples belonging to this species, but construction and use of very specific primers for each species would be difficult and inconvenient for investigation of many specimens belonging to different taxa. Therefore, we asked whether restriction analysis of homologous rDNA fragments may be useful in proper identification of investigated collections.

## Materials and methods

### *Specimens studied*

Samples belonging to different species of genus *Melanelia* were used for identification based on morphological and anatomical features, and as a source of material for DNA isolation. Lichen taxa were identified by W. Fałtynowicz, K. Glanc, M. Kukwa, A. Vězda and B. Guzow-Krzemińska. Voucher specimens are deposited at the University of Gdańsk, Poland.

### *Isolation of DNA*

Extraction of fungal DNA from lichens was based on the modified CTAB method (Armaleo & Clerc, 1995). A piece of thallus (0.25 $cm^2$) was placed in an Eppendorf 1.5 ml tube and ground with a pestle. 0.2 ml of prewarmed (60°C) 2 x CTAB buffer (2% hexadecyltrimethylammonium bromide, 0.7 M NaCl, 50 mM Tris-HCl pH 8.0, 20 mM EDTA, 2% 2-mercaptoethanol) were added, and grinding was continued for several minutes. Following addition of 0.2 ml of prewarmed (60°C) 2 x CTAB buffer and 2 µl of 20 mg/ml proteinase K, the sample was incubated at 55°C for 1 hour. Then RNase I (DNase-free) was added to a final concentration of 10 µg/ml and the incubation was continued at 37°C for 30 min. This mixture was extracted with 0.4 ml of CIA (chloroform-isoamyl alcohol, 25:1). Then, 33 µl of 3 M sodium acetate and 0.2 ml of isopropanol were added to the aqueous phase and nucleic acids were precipitated by incubation for 30 min at -20°C. Following centrifugation (15 min, 11000 *g*) the pellet was washed with 70% ethanol, dried, and resuspended in 30 µl of TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA).

### *PCR amplification*

PCR amplification of rDNA (SSU and LSU) fragments was performed using primers described in Table 1. The Progene (Techne) thermal cycler was employed and following parameters of the PCR reaction were used: an inintial denaturation at 94°C for 4 min, 40 cycles of denaturation at 94°C for 30 s, annealing at 51°C or 52°C for 30 s, primer extension at 72°C for 1 min, and a final incubation for 10 min at 72°C.

Table 1. Oligonucleotide primers used for amplification of fragments of fungal rDNA

| Primer | Nucleotide sequence (5'→3') | Coordinates of *Saccharomyces cerevisiae* rDNA | Reference |
|---|---|---|---|
| BG | TGC TTA ATT GCG ATA ACG | 1304-1321 SSU rDNA | This work |
| 9 | AAT GAT CCT TCC GCA GGT | 1797-1780 SSU rDNA | DePriest & Been, 1992 |
| LR0R | GTA CCC GCT GAA CTT AAG | 26-42 LSU rDNA | Vilgalys, 2000 |
| LR7 | TAC TAC CAC CAA GAT CT | 1448-1432 LSU rDNA | Vilgalys & Hester, 1990 |

*Restriction analysis of PCR products*

For restriction analysis, 15 µl of each PCR product was digested in total volume of 20 µl, using *Hae*III (*Bsu*RI) or *Alu*I enzymes (Fermentas) under conditions recommended by the manufacturer. Reaction products were separated by 2% agarose gel electrophoresis according to Sambrook *et al.* (1989). The 100 bp DNA Ladder Plus (Fermentas) was used as a molecular size marker. After ethidium bromide staining, the gels were photographed over a UV transilluminator (312 nm wavelength).

## Results

In order to use restriction analysis of PCR-amplified DNA fragments as a feature in proper identification of lichens, the same primers should be used for all taxa tested. For example, if one wants to test collections from a particular genus, each pair of primers should be appropriate for amplification of particular homologous DNA fragment from all species of this genus. As a model, we investigated species from genus *Melanelia*. The pairs of primers were designed to amplify a fragment of the region coding for the small subunit ribosomal RNA (SSU) or the large subunit ribosomal RNA (LSU). The primers were complementary to conservative regions between which the variable region was located. Therefore, using one pair of primers, we were able to amplify the particular homologous region of DNA from samples belonging to different species, encompassing the variable region flanked by two conservative DNA sequences.

In the case of the SSU region, the differences between *Melanelia* species appeared even after PCR amplification (Figs. 1 and 2). However, much more characteristic patterns for each species appeared after digestion of the PCR products with specific restriction enzymes (Figs. 1 and 2). In most cases, each species can unambiguously be distinguished from others on the basis of the restriction pattern. If the patterns are similar for two (or more) species, e.g. *M. subaurifera* and *M. subargentifera* in Fig. 1, using other restriction enzyme(s) should allow to distinguish the species.

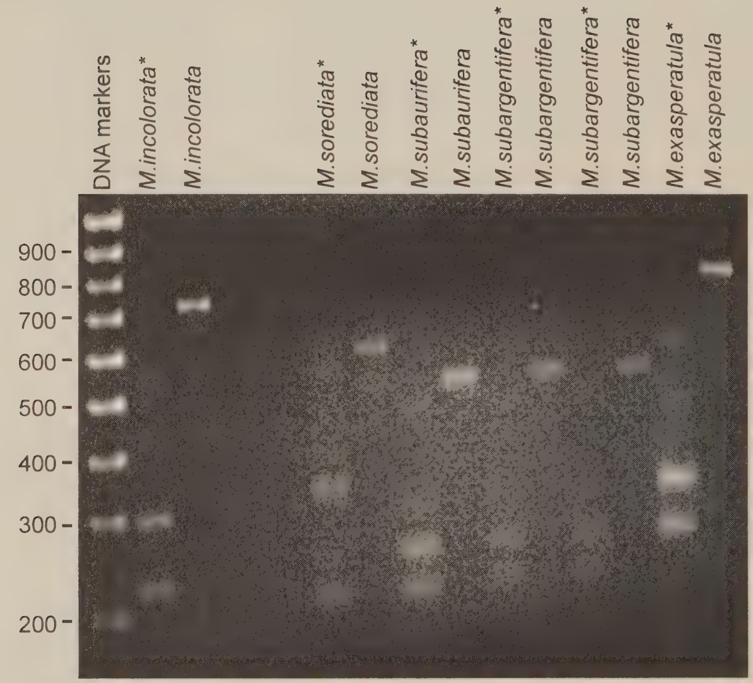

Fig. 1. Restriction fragment length polymorphism (RFLP) of SSU rDNA region from different species of genus *Melanelia*. The DNA regions amplified by PCR with primers BG and 9 (see Table 1) were digested with *Alu*I restriction enzyme. DNA fragments were subjected to 2% agarose gel electrophoresis. Sizes of DNA markers (in base pairs) are depicted. Each lane, except that with molecular size markers, is described by the name of species. The lanes with the names marked with asterisks represent DNA samples digested with the restriction enzyme, and other lanes represent undigested samples of PCR products.

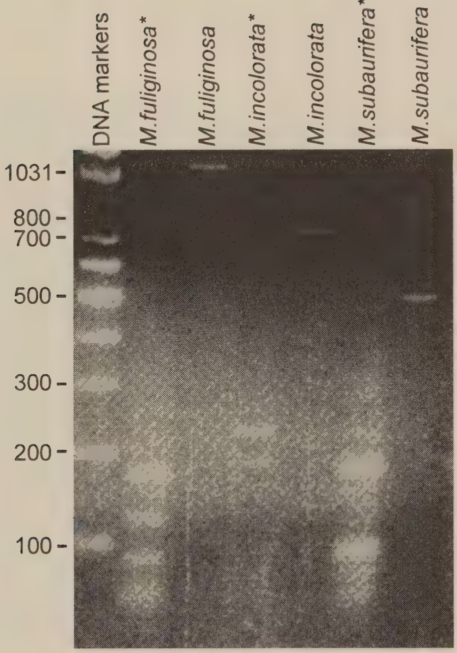

Fig. 2. Restriction fragment length polymorphism (RFLP) of SSU rDNA region from different species of genus *Melanelia*. The DNA regions amplified by PCR with primers BG and 9 (see Table 1) were digested with *Bsu*RI (a *Hae*III isoschizomer) restriction enzyme. DNA fragments were subjected to 2% agarose gel electrophoresis. Sizes of DNA markers (in base pairs) are depicted. Each lane, except that with molecular size markers, is described by the name of species. The lanes with the names marked with asterisks represent DNA samples digested with the restriction enzyme, and other lanes represent undigested samples of PCR products.

Investigating the LSU regions, we found no significant differences in the length of PCR-amplified DNA fragments from different species (Fig. 3). However, restriction patterns of DNA from particular species were considerably different (Fig. 3). Moreover, we found that a few alternative *Bsu*RI restriction patterns occurred in the same species, e.g. three for *M. exasperata* and two for *M. exasperatula* (Fig. 3). Nevertheless, it is worth noting that we found no example of the same restriction pattern from samples belonging to different species. Therefore, although in some cases more than one restriction patterns were observed among samples from one species, all these patterns were characteristic and specific for this species.

## Discussion

Many taxonomical problems may be potentially solved by using molecular biology techniques. However, until now these techniques were used mostly for determination of relationships between different taxa and for identification of one species among the others. Here we asked whether molecular biology techniques may be used for establishment of features recognized as suitable in the identification keys. DNA sequencing, although very informative, is currently too expensive and too time consuming procedure for analysis of many samples. On the other hand, construction and usage of specific primers, different for each species, is inconvenient in such studies. Therefore, we investigated restriction fragment length polymorphism (RFLP) of PCR-amplified rDNA fragments.

Using primers complementary to conserved regions that flanked a variable region, we were able to amplify homologous DNA sequences from different species with just one pair of oligonucleotides. Our studies on several species from genus *Melanelia* revealed that it is possible to obtain restriction patterns characteristic for each species. It is very important to note that for identification purposes, finding one and only one restriction pattern with certain enzyme for one species is not necessary. For example, there may be three different restriction patterns present among collections belonging to one species, but as long as they are characteristic only to representatives of this species (and not to those of other species), they may serve as identification features.

Therefore, we propose that identification keys of lichens based on restriction analysis of PCR-amplified DNA fragments can be constructed. For such keys, construction of a set of restriction patterns of particular DNA regions for all investigated species is necessary. Examples of fragments of such sets are presented in Tables 2 and 3. Having this information, molecular biology-based identification keys may be constructed. We suggest that the identification keys based on RFLP data should not be alternative to classical keys. They should rather be accessory keys, used when proper identification of particular collections is difficult on the basis of morphological and anatomical purposes.

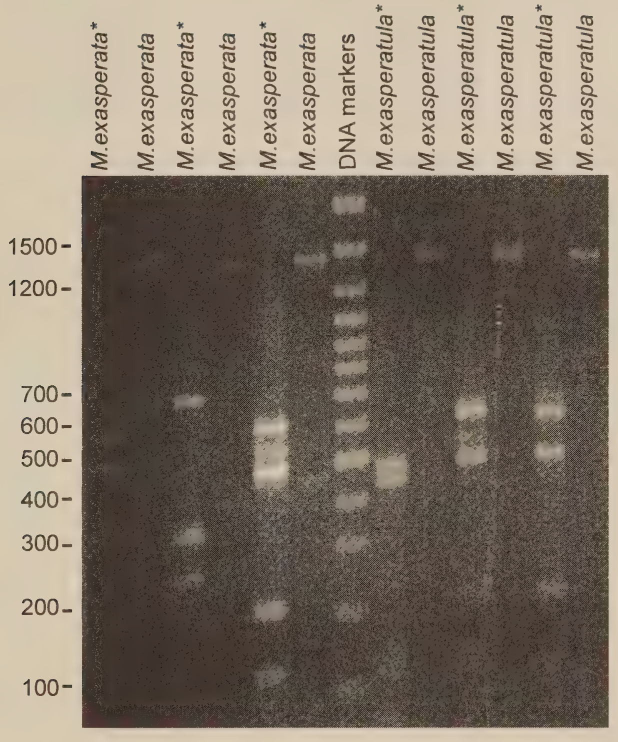

Fig. 3. Restriction fragment length polymorphism (RFLP) of LSU rDNA region from different species of genus *Melanelia*. The DNA regions amplified by PCR with primers BG and 9 (see Table 1) were digested with *Bsu*RI (a *Hae*III isoschizomer) restriction enzyme. DNA fragments were subjected to 2% agarose gel electrophoresis. Sizes of DNA markers (in base pairs) are depicted. Each lane, except that with molecular size markers, is described by the name of species. The lanes with the names marked with asterisks represent DNA samples digested with the restriction enzyme, and other lanes represent non-digested samples of PCR products.

Table 2. A fragment of a set of restriction patterns characteristic for different species from genus *Melanelia* after amplification of a fragment of SSU rDNA region using primers BG and 9 (see Table 1). Only major DNA fragments are listed.

| Species | Length of PCR product (base pairs) | Restriction enzyme | Characteristic restriction fragments (base pairs) |
|---|---|---|---|
| *M. exasperatula* | 820 | *Alu*I | 380, 290, 170 |
| *M. incolorata* | 720 | *Alu*I | 300, 210 |
| *M. sorediata* | 620 | *Alu*I | 330, 210 |
| *M. subaurifera* | 530 | *Alu*I | 260, 210 |
| *M. subargentifera* | 540 | *Alu*I | 270, 210 |
| *M. fuliginosa* | 1100 | *Bsu*RI (*Hae*III) | 180, 130, 80, 50 |
| *M. incolorata* | 720 | *Bsu*RI (*Hae*III) | 220, 180 |
| *M. subaurifera* | 530 | *Bsu*RI (*Hae*III) | 180, 90 |

Table 3. A fragment of a set of restriction patterns characteristic for different species from genus *Melanelia* after amplification of a fragment of LSU rDNA using primers LR0R and LR7 (see Table 1). Only major DNA fragments are listed.

| Species | Length of PCR product (base pairs) | Restriction enzyme | Characteristic restriction fragments (base pairs) |
|---|---|---|---|
| *M. exasperata* | 1450 | *Bsu*RI (*Hae*III) | Pattern I:<br>540, 440, 290, 180<br>Pattern II:<br>700, 320, 250<br>Pattern III:<br>600, 480, 200, 110 |
| *M. exasperatula* | 1450 | *Bsu*RI (*Hae*III) | Pattern I:<br>490, 450, 120, 80<br>Pattern II:<br>650, 500, 220, 130 |

## Acknowledgments

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# STROPHARIA ALBIVELATA AND ITS BASIONYM PHOLIOTA ALBIVELATA

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*Abstract: Pholiota albivelata* is transferred to *Stropharia* following the documentation of acanthocyte formation on its mycelium, and the name *Stropharia earlei* is proposed for *Pholiota cubensis.*

*Key words:* Agaricales, Basidiomycetes, BLM, PNW Forest Plan, Strophariaceae

Since the dark-spored Pacific Northwest endemic fungus *Pholiota albivelata* Murr. was described almost ninety years ago (Murrill, 1912), this relatively infrequently encountered agaric has been documented from humic soils in wet coniferous Pacific Northwest forests (Stuntz & Isaacs 1962, Smith & Hesler 1968) and listed as a "Survey and Manage Strategy 1" fungus as federally mandated by the Pacific Northwest Forest Plan (USDA/DI 1993, 1994ab, Castellano et al. 1999). Two characters -- production of a brown spore deposit and possession of an annulus -- prompted Murrill to place his new species in *Pholiota,* a classification that either has been accepted (Overholts 1924, 1927) or accepted with reservations (Smith & Hesler 1968; Singer 1975, 1986). Inclusion of the species in *Pholiota* has influenced the way that genus has been characterized in modern monographs. For example, Smith & Hesler (1968) specifically cited *P. albivelata* as an example of a species with a membranous persistent annulus (p. 14), even while stressing that "The *P. albivelata* group connects obviously to *Stropharia*..... (p. 30)" In the same text, the two authors described a new section in subgenus *Phaeonaematoloma* (Singer) Singer (*i.e.* Sect. Albivelatae, typified by *P. albivelata* and containing two stirps, "Albivelata" and "Cubensis"). Smith (1979) later commented on the exceedingly close relationship between *Stropharia* and *P. albivelata* (separated only by spore color) in his discussion on generic concepts. Singer, who accepted the section Albivelatae in *Pholiota*, noted that it was in this section of *Pholiota* "...where spore print colors vary most, thus where 'transitions' to *Stropharia* [occur]...." (Singer 1975: 552, 1986: 581).

Recently in Oregon both authors -- the senior while identifying mushrooms as guest mycologist at the 1999 Oregon Mycological Society Fall Foray in Marion County and both during a "Survey and Manage" research inventory in Benton County -- had the opportunity to examine fresh collections of *Pholiota albivelata.* (In the latter instance, the specimens were mistaken as representing a *Stropharia* near *S. hornemannii* (Fr.:Fr.) Lundell & Nannfeldt.) Both collections, which were examined for the presence of distinctive calcium oxalate-capped stellate cells (acanthocytes) characteristic of *Stropharia* (Farr 1980, Redhead 1984ab, Jahnke 1984), possessed abundant acanthocytes on both mycelia and mycelial cord surfaces. Hence we consider *P. albivelata* to represent *Stropharia* rather than *Pholiota* and accordingly propose its transfer to *Stropharia* as:

***Stropharia albivelata*** (Murrill) Norvell & Redhead, ***comb. nov.*** (basionym: *Pholiota albivelata* Murrill, *Mycologia* 4: 260. 1912).

When recognized in Europe as a distinct genus, *Stropharia* is considered to contain species that produce either purple-brown or tobacco brown spore deposits (Moser 1968, Watling & Gregory 1987). Based as it was upon spore deposit color, the generic delimitation for *Pholiota* used by Smith & Hesler (1968) has been much criticized by other authors (Bas 1971, Singer 1975, 1986). In point of fact, Smith -- who had not placed such over-riding importance on spore color in earlier publications (Smith 1951, Singer & Smith 1946) -- appears to have adopted the simpler Friesian concept for convenience during preparation of the *Pholiota* monograph. Unfortunately, the inflexible delimitation of

*Pholiota* resulting from over-emphasizing the brown spore print resulted in (i) the artificial inclusion in *Pholiota* of such taxa as *Phaeolepiota aurea* (Fr.) Konrad & Maublanc, (ii) misdiagnosis of the true affinity of *Pholiota fulvosquamosa* Peck (a synonym of *Agaricus subrufescens* Peck, *cf.* Redhead 1984b), and (iii) generation of a superfluous species name (*Pholiota subcaerulea* Smith & Hesler) for a species of *Stropharia* known previously from Europe, *S. pseudocyanea* (Desm.:Fr.) Morgan (as noted by Redhead 1984a, *cf.* also Watling & Gregory 1987). Therefore it is not too surprising to discover another misplaced *Stropharia* among the oddly circumscribed taxa.

At least one other species in Sect. *Albivelatae* -- *Pholiota cubensis* Earle -- is also a *Stropharia.* The junior author's examination of an isotype at the University of Michigan herbarium (MICH) revealed the presence of acanthocytes. Because the name *Stropharia cubensis* is preoccupied by *Stropharia cubensis* Earle (a different and unrelated species currently accepted as *Psilocybe cubensis* (Earle) Singer), we propose the new name:

***Stropharia earlei*** Norvell & Redhead, ***nom. nov.*** (basionym: *Pholiota cubensis* Earle, Inf. An. Estac. Centr. Agron. Cuba 1: 242. 1906).

We have not examined the type of *Pholiota sipei* Smith & Hesler but feel strongly that it represents a *Stropharia.* Singer (1975, 1986) twice listed it as a suspected species of *Stropharia,* and Smith & Hesler (1968) themselves noted that were it not for the spore color of *P. sipei* "...one would immediately place the species in *Stropharia...*" Given (i) that no one has rediscovered *P. sipei,* (ii) that its description combines features from two endemic PNW species -- *S. albivelata* with a brownish spore deposit and *S. hornemannii* having the same dual types of pleurocystidia (Smith 1949) and same spore size range as described for *P. sipei* (Smith & Hesler 1968) -- and (iii) that Smith & Hesler (1968) appear to have relied upon secondhand observations when describing a species from only one collection, we suspect that *P. sipei* may not be a good species.

We also have not examined the fourth species included in Section *Albivelatae* by Smith and Hesler (1968) --*Pholiota johnsoniana* (Peck) Atkinson -- but note that in 1962 Singer treated it as *Stropharia johnsoniana* (Peck) Peck. Smith and Hesler (1968) admitted that its purple-brown spore coloration justifies its placement in either *Pholiota* or *Stropharia.* While discussing *Stropharia,* Singer (1975, 1986) later specifically addressed several "ambiguous" species such as *P. johnsoniana* and agreed with Smith and Hesler's 1968 disposition of the species in *Pholiota* (although he -- somewhat ironically -- failed to list the species in either genus in both editions of his monograph). Singer (1975: 531, 1986: 560) chose to accept Smith and Hesler's disposition of *P. johnsoniana* in *Pholiota* rather than in *Stropharia* in part because "...it can be avoided that an otherwise continuous series between that species and *Pholiota* section *Albivelatae* [would have] to be broken and divided between the genera *Stropharia* and *Pholiota."* Our recent discovery of acanthocytes in *S. albivelata* would indicate that Singer ought to have retained *P. johnsoniana* in *Stropharia*. In view of the above, we suggest that the Section *Albivelatae* is, in fact, an artificial taxon misplaced in *Pholiota*. While some contemporary authors (*e.g.* Noordeloos 1995, Stamets 1996) might consider it more appropriate to transfer the species to an expanded concept of *Psilocybe* (including *Stropharia* and *Pholiota)*, we view such a solution as premature in light of recent DNA data analysis of the Strophariaceae available to the junior author. Hence we opt to retain *Stropharia.*

## *Specimens Examined*

(PNW-MS designates the Pacific Northwest Mycology Service Herbarium)

***Stropharia albivelata.*** UNITED STATES OREGON: Benton Co -- BLM Green Peak Density Study: in leafy duff under *Acer macrophyllum, Pseudotsuga menziesii 24.XI.1999* GP1991124U1-02 RLExeter, LLN, SAR (PNW-MS, DAOM); Marion Co -- Road to Opal Lake: *23.X.1999* LLN1991023-32 LGilpin, RCarlson (PNW-MS).

***Stropharia ambigua.*** UNITED STATES OREGON: Benton Co -- BLM Green Peak Density Study: in leafy duff under *Acer circinatum, Pseudotsuga menziesii 24.XI.1999* GP1991124U2-02 SAR, LLN (PNW-MS).

***Stropharia earlei.*** CUBA: Prov. De la Habana, Estacion Central Agronomica, on ground, *31.VIII.1904* FSEarle 159 by AMechleff (isotype of *Pholiota cubensis* Earle, MICH).

***Stropharia hornemannii:*** UNITED STATES OREGON: Marion Co -- Road to Opal Lake, *23.X.1999* LLN1991023-33a KPatrick (PNW-MS), LLN1991023-33b JKelly, ZKopecky (PNW-MS).

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# NOTES ON WESTERN NORTH AMERICAN SNOWBANK FUNGI

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**ABSTRACT** -*Lyophyllum montanum* AH Smith is transferred to *Clitocybe* as *Clitocybe glacialis* (non *C. montana* Harmaja). It lacks the siderophilous granules characteric of *Lyophyllum*. The synonymy of *Hygrophorus angelesianus* and *Clitocybe mutabilis* is confirmed by type studies. *Neohygrophorus angelesianus* is accepted as the correct name and the new combinations *N. cokeri* (= *Clitocybe cokeri*) and *N. umbrino-purpurascens* (= *Clitocybe umbrino-purpurascens*) are proposed. A key to *Neohygrophorus* is provided.

**KEY WORDS:** USA, Pacific Northwest, Appalachians, Cascades, Rockies, subalpine, Atlas Mountains, Algeria, Canada, Hygrophoraceae, Tricholomataceae.

## 1. *Clitocybe glacialis / Lyophyllum montanum*

For western North America, the genus *Lyophyllum* is generally represented in field guides (e.g. Smith 1975, Arora 1986, McKnight & McKnight 1987) by two common species -- *Lyophyllum decastes* (Fr.) Singer and *L. montanum* A.H. Smith. The former actually represents a species complex commonly encountered in urban and rural areas (Moncalvo & Clémençon 1992, Moncalvo et al 1993). The latter is a snowbank species commonly found in western alpine and subalpine habitats (Miller 1967, Evenson 1997) that some teachers have used to demonstrate the genus *Lyophyllum* to their students. Unfortunately, attempts to illustrate the most significant *Lyophyllum* generic character -- revealing siderophilous (carminophilous) granules in basidia using acetocarmine (Clémençon 1978) -- repeatedly have proven futile, resulting in researchers and students alike questioning either the viability of their acetocarmine solution or the utility of the acetocarmine staining procedure in general. No such difficulties have been encountered, however, when specimens representing the *L. decastes* complex have been tested.

We have made numerous collections of *Lyophyllum montanum* in subalpine and alpine habitats. Repeated testing of both fresh and dried samples failed to demonstrate the presence of siderophilous granules in basidia. Similarly the holotype of *L. montanum* borrowed from the University of Michigan (MICH) also lacks siderophilous granules in the basidia and does not have siderophilous basidiospore walls. (Our specimens were also shown to match the type in all other significant respects). Hence, as currently defined, the common western North American snowbank species, "*Lyophyllum*" *montanum* is not a *Lyophyllum*. In a genus defined by a cytological feature, this anomaly requires some explanation.

The type was originally collected from Wyoming's Medicine Bow Mountains by Dr. Harry Thiers, who later gave it to Alex Smith. Smith (1957) apparently assumed that the collection represented an unnamed *Lyophyllum* because of its dark grey coloration and lamellae that became more cinereous with age. At that time, several other mycologists working in western North America were also familiar with the species, which was commonly collected near melting snowbanks at higher elevations in the spring; Cooke (1955a, b), for instance, reported a common grey unidentified snowbank '*Lyophyllum*' from western subalpine areas in California, Oregon, Washington, Utah, and Wyoming (including the Medicine Bow range). There is no indication in either Smith's original description or any collection notes that he ever attempted to stain the type specimens with acetocarmine, nor has there any additional report that anyone else has demonstrated the presence of siderophilous granules. (It is noteworthy that in the same paper, Smith (1957) neglected to report on the presence of such granules in his descriptive note on *L. rancidum* (Fr.) Singer, even though it has been shown by others to possess them. Thus we consider Smith's omission a simple oversight.)

At the present time, the genus *Clitocybe*-- in a moderately broad sense, although not as broad as proposed by Bigelow in 1982 and 1985 -- would appear to be the only genus that might accommodate *"Lyophyllum" montanum*. Several alpine and subalpine species (including *C. festiva* Favre and *C. mortuosa ss* Favre 1955) have similarly dark pigments, possibly developed as protection against ultraviolet radiation in rarefied atmospheres. Unfortunately because the name "*Clitocybe montana*" has already been used (Harmaja 1976), a new name must be coined for the *"Lyophyllum montanum"* transferred to *Clitocybe*. Therefore we propose the following:

***Clitocybe glacialis*** Redhead, Ammirati, Norvell & Seidl, **nom. nov.**
≡*Lyophyllum montanum* Smith, Beih. Sydowia 1: 60. 1957.
non *Clitocybe montana* Harmaja, Karstenia 15: 19. 1976.

## 2. *Neohygrophorus angelesianus / Clitocybe mutabilis*

A second common snowbank agaric found in western North America is *Neohygrophorus angelesianus* (Smith & Hesler) Singer. Like *C. glacialis*, it produces darkly pigmented basidiomes that melt their way through receding alpine and subalpine snow packs in spring and early summer (Miller 1965, 1967). Originally described as a *Hygrophorus* by Smith & Hesler (1942), the species was placed into its own subgenus [*Hygrophorus* subgenus *Pseudohygrophorus* Smith & Hesler (nom. nud.)] to accommodate the combination of amyloid spores with pigments in pileal and stipe tramal tissues that change from grey to red in 2% KOH solution (cf also Hesler & Smith 1963). When recognized in a separate genus, it has been placed in the monotypic *Neohygrophorus* Singer.

By 1993 many mycologists, including junior authors Ammirati and Seidl as well as Dr. Orson Miller, were aware that Bigelow had applied a second name --

*Clitocybe mutabilis* Bigelow (1977, 1985) -- to this same species (in 1995 Bessette et al. published this synonymy and included a color photograph of the species.) Aware of a potential synonymy and knowing that Bigelow (1977, 1985) had also placed *Clitocybe mutabilis* with one other species -- *Clitocybe cokeri* Hesler in Smith & Hesler (1943) -- into *Clitocybe* subgenus *Mutabiles* Bigelow, we borrowed type specimens for both *Hygrophorus angelesianus* and *Clitocybe mutabilis* from the University of Michigan (MICH) and the type and other specimens of *C. cokeri* from the University of Tennessee (TENN).

Our morphological and anatomical research confirmed that the *Hygrophorus* (now *Neohygrophorus*) *angelesianus* and *C. mutabilis* type specimens represented the same species. Among other features, both had amyloid spores within nearly the same size range and tramal tissues that conspicuously changed colors in 2% KOH solution. Pigments in the *Hygrophorus angelesianus* type were both intraparietal and intracellular, reddish to yellowish brown in water, becoming immediately purplish to vinaceous in KOH solution and then fading within minutes; pigments in the *Clitocybe mutabilis* type were also reddish to yellowish brown, becoming greyish vinaceous in KOH and then fading rapidly. Both likewise possessed a gelatinized pileipellis. Our examinations also revealed that *C. cokeri* had virtually the same combination of features (with tissues very similar to those in *N. angelesianus*): tramal pigments in the *C. cokeri* type were incrusting to intraparietal, yellowish to orangish brown in water, becoming immediately salmon to rose tinted in KOH and then fading within minutes. Its gelatinized pileipellis, however, contained scattered thin orangish refractive hyphae with contents that did *not* fade in KOH. Hence, although the tissues were similar enough to justify placement in the same genus, the *C. cokeri* specimen differed from the other two types significantly enough to merit its recognition as a separate species. Bigelow (1985) distinguished the two species by size and odor/taste (noting that *C. cokeri* produced larger basidiomes characterized by a farinaceous odor and taste) as well as by their disjunct distribution (*Clitocybe cokeri* appears to be restricted to Tennessee in the Great Smoky Mountains and near Knoxville whereas *N. angelesianus* (as *C. mutabilis*) is a western montane species. Based upon these considerations and our examination, we believe that *C. cokeri* is best treated as a second species in the genus *Neohygrophorus*:

***Neohygrophorus cokeri*** (Hesler) Redhead, Ammirati, Norvell & Seidl, **comb. nov.**

≡ *Clitocybe cokeri* Hesler in Smith & Hesler, Lloydia 6: 251. 1943.

The distribution and ecology of *N. cokeri* merit further comment. Bigelow

(1985) and Smith & Hesler (1943) noted [specifically from TENN specimens Dec 1, Dec 15, Dec 19, Dec 24, Jan 10, Jan 11 and Jan 25] that the species fruited during the two coldest months -- January and December. This would indicate that it -- like its sister taxon *N. angelesianus* -- is a cold tolerant species. The geographic separation between these two sister species recalls the disjunct distribution of another western subalpine snowbank agaric, *Mycena griseoviridis* A.H. Smith (1947), also collected from near western mountain snowbanks (Cooke 1944, 1955a, b) and from eastern North America (found from Quebec to Michigan and southwards along the Appalchians into Tennessee (Smith 1947) and North Carolina (demonstrated to SAR by Rytas Vilgalys), where it fruits during cold winter months. More restricted is the non-agaric, *Gomphus kauffmanii* (Smith) Petersen (1971), originally described from the pacific northwest states but also known from Connecticut, Tennessee and North Carolina. From such disjunct distributional patterns (cf. also Redhead 1989), it would appear that the species pair *Neohygrophorus angelesianus* / *N. cokeri* represent divaricant taxa.

Interestingly, Smith & Hesler (1943) reported that *C. cokeri* occurred in Tennessee and Oregon. The Oregonian report appears based on an anomalous collection (AHS 14149 from Sisters OR, 29 Oct. 1941) containing specimens bearing lilac-colored mycelium. The fact that Bigelow (1985) later described *N. angelesianus* (*as C. mutabilis*) as having a lilac colored mycelium that *C. cokeri* lacked, suggests that the Oregon collection cited in the protologue for *C. cokeri* probably represents *N. angelesianus*, particularly as when one considers that the latter species was subsequently (and independently) reported by Miller (1965) from Oregon's Three Sisters Wilderness area. It is noteworthy that the anomalous collection was made in October, rather than the spring. While conducting a survey of fungi in a lower elevation (1,200 ft elev.) young *Pseudotsuga menziesii* (Mirb.) Franco forest in Washington state, *N. angelesianus* was encountered twice in the late fall (e.g. Pack Forest, WA, 8 Nov. 1986, S. Ammirati 319 WTU). Therefore, it appears that *N. angelesianus* is neither totally restricted to high elevations near snowbanks, nor to vernal fruitings.

Also noteable are the facts that (i) neither Smith nor Hesler recognized the congeneric nature of two species they described separately one year apart and (ii) Bigelow described *N. angelesianus* a second time while overlooking his previous Oregon record under the name *C. cokeri*. In our experience, problematic species that do not easily match described genera frequently end up being described as new species more than once. *Clitocybe cokeri* is not typical for either *Hygrophorus* or *Clitocybe*, and while Singer's (1951, 1961) taxonomic solution -- recognizing *Neohygrophorus* as a separate distinct genus (cf. also Horak 1968) -- partly addresses the problem, difficulties with family placement (with *Clitocybe* in the Tricholomataceae and *Hygrophorus* / *Neohygrophorus* in the Hygrophoraceae) could be expected to complicate initial identification of the species now treated in *Neohygrophorus*.

Bigelow's *(1985)* synonymization of *Clitocybe umbrino-purpurascens* R. Maire (1945) with *N. cokeri* (as *C. cokeri*) extends the distribution of *Neohygrophorus* into North Africa. We cannot, however, accept Bigelow's proposed synonymy. Bigelow (1985) separated *N. cokeri* from *N. angelesianus* (as *C. mutabilis*) in part based upon odor, taste and size [expressed as stipe length] (in addition to the lilac colored mycelium) with *N. cokeri* producing larger stipes (3-8 cm long) and farinaceous taste and odor. Maire (1945), who collected his in the fall under *Cedrus atlantica* (Endl.) G. Manetti above Blida (presumably in the Atlas mountains), Algeria, described smaller basidiomes with shorter stipes (1.5-3 cm, within the range cited for *N. angelesianus*) having a bitter, rather than farinaceous, taste. Hence, we believe it represents a third *Neohygrophorus* species for which we propose the name:

***Neohygrophorus umbrino-purpurascens*** (R. Maire) Redhead, Ammirati, Norvell & Seidl, **comb. nov.**

≡ *Clitocybe umbrino-purpurascens* R. Maire, Bull. Soc. Hist. Natur. Afric. Nord 36: 35. 1945.

## Key to *Neohygrophorus*

**1.** Odor farinaceous (fruiting in fall or winter, eastern USA or N. Africa) . . **2**

**1.** Odor not distinctive (spring or summer fruiting near high elevation snowbanks in western North America; or late fall at lower elevations) . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . ***N. angelesianus***

**2.** Taste bitter, from N. Africa under *Cedrus atlantica* (fall fruiting) . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . ***N. umbrino-purpurascens***

**2.** Taste farinaceous, from southeastern USA (winter fruiting) . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . ***N. cokeri***

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# BAEODROMUS RANUNCULI, A NEW RUST ON RANUNCULUS FROM ARGENTINA, AND A SYNOPSIS OF BAEODROMUS

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**ABSTRACT:** *Beaodromus ranunculi* is described as a new species on *Ranunculus flagelliformis* from Argentina. Nomenclatural, host, and distribution information is provided for the seven other species described in *Baeodromus,* as well as a key to the accepted species.

**Key Words:** Argentina, *Baeodromus*, new species, Pucciniosiraceae, Ranunculaceae, *Ranunculus*, rust, systematics, Uredinales.

*Baeodromus* Arthur (1905) is one of a group of microcyclic rusts considered to have a polyphyletic origin, derived mostly from long-cycled species by the endophylloid pathway of life cycle reduction (Buriticá & Hennen, 1980). Although Azbukina (1972) established the tribe Baeodromae in the family Melampsoraceae Schroet. for this genus, Buriticá & Hennen (1980) placed *Baeodromus* in the tribe Pucciniosireae Dietel (1928) and included other microcyclic genera such as *Endophyllum* Lév., *Pucciniosira* Lagerh., and *Dietelia* Henn. Cummins and Hiratsuka (1983) established the family Pucciniosiraceae (Dietel) Cummins & Y. Hirats., in which *Baeodromus* is now placed. Some genera in this family produce spermogonia that, when present, are Type IV, Group V (per Hiratsuka & Hiratsuka, 1980), and the telia (*Aecidium*-like or derived structures) are compact, with or without a peridium. The teliospores are 0-1-septate, catenulate, hyaline, but mostly lightly pigmented, with intercalary cells that sometimes are modified to form a pedicel-like structure. This family includes the genera *Alveolaria* Lagerh., *Baeodromus, Chardoniella* F. Kern, *Cionothrix* Arthur, *Didymopsora* Dietel, *Dietelia, Pucciniosira* and *Trichopsora* Lagerh. Buriticá (1991) also included *Ceratocoma* Buriticá & J.F. Hennen in this family.

*Baeodromus* includes species in which the life cycle is reduced to a telial stage or telial and spermogonial stages (Buriticá, 1991; Buriticá & Hennen, 1980; Cummins & Hiratsuka, 1983). Telia are erumpent with no peridia and the teliospores are catenulate or organized irregularly in chains, and adherent laterally and vertically resulting in a compact mass. The teliospores are non-septate but sometimes appear 1-septate. They germinate without dormancy. When present, spermogonia are subepidermal and globoid and placed in Type IV, Group V, according to Hiratsuka and Hiratsuka (1980).

Seven species have been placed in *Baeodromus*. Buriticá (1991) included five species, of which four were from the Americas on *Senecio* (Asteraceae) and one from Russia on *Urtica* (Urticaceae). *Baeodromus dominica* (F. Kern) Buriticá & J.F. Hennen on *Croton* (Euphorbiaceae) was transferred to *Uredopeltis* Henn. by Buriticá and Hennen (Buriticá, 1998). *Baeodromus eupatorii* (Arthur) Arthur, not treated by Buriticá (1991), occurs on *Eupatorium* in Mexico and Central America. Based on the literature and databases associated with the U.S. National Fungus Collections a nomenclator is presented with host and distribution information for the seven species.

***Baeodromus albertensis*** Buriticá, Rev. Acad. Colomb. Cienc. 18(69): 145. 1991. Type: on *Senecio eremophilus* Richardson (Asteraceae) from Canada, Alberta, near Delta, 8 July 1940, *A.H. Brinkman 5017* (holotype PUR 49724; isotype DAOM 6778). Referred to as *Baedromus* sp. in Cummins (1978, p. 389).

The Index of Fungi (I.M.I., 1992) indicated that *B. albertensis* was not validly published because more than one specimen was listed as the type (ICBN Art. 37). Actually only one gathering was listed (*A.H. Brinkman 5017*) but specimens at two herbaria (PUR and DAOM) were cited. Based on Proposal 80 to amend Article 37.3 of the Code (Barrie, 1998), which was approved at the XVI International Botanical Congress (Barrie & Greuter, 1999), this name is valid. That amendation states "...for the name of a new species or infraspecific taxon, citation of a single gathering or illustration is acceptable as indication of a type..." Buriticá (pers. comm.) based his description on the specimen at PUR, therefore according to ICBN Rec. 9A.4 (Greuter et al, 1994) that specimen should be considered the holotype. Therefore, the name *Baeodromus albertensis* is considered valid because all the other requirements for valid publication were met by Buriticá (1991).

Hosts and distribution: on *Senecio eremophilus*, Canada.

***Baeodromus californicus*** Arthur, Ann. Mycol. 3:19. 1905. Holotype: on *Senecio douglasii* DC. (Asteraceae), from USA, California, San Bernardino, Little Creek, 25 April 1885, *S.B. Parish 2562* (PUR 5785).

Hosts and distribution: on *Senecio douglasii*, USA.

***Uredopeltis dominicana*** (F. Kern) Buriticá & J.F. Hennen, Rev. Acad. Colomb. Cienc. 22:330. 1998.

≡ *Baeodromus dominicana* (F. Kern) Thirum. & F. Kern, Mycologia 41:284. 1949.

≡ *Phakopsora dominicana* F. Kern, Mycologia 20:63. 1928. Type: on *Croton angustatus* Urban (Euphorbiaceae) from Dominican Republic, Santiago, San José de las Matas, 8 May 1927, *Chardon 397* (PUR 44006).

Hosts and distribution: on *Croton angustatus*, Dominican Republic; on *Croton ciliatoglandulifer* Ortega, Mexico.

***Baeodromus eupatorii*** (Arthur) Arthur, N. Amer. Fl. 7:125. 1907.

≡ *Dietelia eupatorii* Arthur, Bot. Gaz. 40:197. 1905. Holotype: on *Eupatorium pazcuarense* Kunth (Asteraceae) from Mexico, State of Mexico, Amecameca, 21 Oct 1903, *Holway 5205* (PUR 5777).

Hosts and distribution: on *Eupatorium adenophorum* Spreng., Mexico; on *Eupatorium aschenbornianum* Schauer, Guatemala, Honduras; on *Eupatorium mairetianum* DC., Guatemala; on *Eupatorium pazcuarense*, Guatemala, Mexico; on *Eupatorium pycnocephalum* Less., Guatemala; on *Eupatorium* sp., Guatemala, Mexico.

***Baeodromus holwayi*** Arthur, Ann. Mycol. 3:19. 1905. Type: on *Senecio cinerarioides* Kunth (Asteraceae) from Mexico, Nevado de

Toluca, 15 Oct 1903, *Holway 5160* (holotype PUR 5780; isotypes Bartholomew – N. Amer. Uredinales 207).

Hosts and distribution: on *Senecio argutus* Kunth, Mexico; on *Senecio cinerarioides*, Mexico; on *Senecio warszewiczii* Braun & Bouche, Guatemala (Cummins, 1978).

***Baeodromus senecionis*** Syd., Monographia Uredinearum 3:549. 1915. Type: on *Senecio* sp. (Asteraceae) from Ecuador, Chimborazo, Yerbabuena, Sept 1891, *Lagerheim* (isotype PUR F8722).

Hosts and distribution: on *Senecio betonicaefolius* DC.; Ecuador; on *Senecio* sp., Ecuador.

***Baeodromus tranzschelii*** Azbukina, Nov. Syst. Pl. Non Vasc. 7:230. 1970. Type: on *Urtica laetevirens* Maxim. (Urticaceae) from Russia, Primorskensis, Ternejskij, 10 July 1957, *Z.M. Azbukina* (LE). [*Baedromus urticae* Tranzschel, ad interim, Tranzschel, Cons. Ured. URSS, Moscow, p. 62. 1939. (*nom. nud.*).Type: on *Urtica laetevirens* Maxim. from Russia, Far East, Maritime Territori, in the mountains above the Kharitonovka River, 14 Sept 1929, *Tranzschel s.n.*]

Hosts and distribution: on *Urtica laetevirens* Maxim., China, Russia.

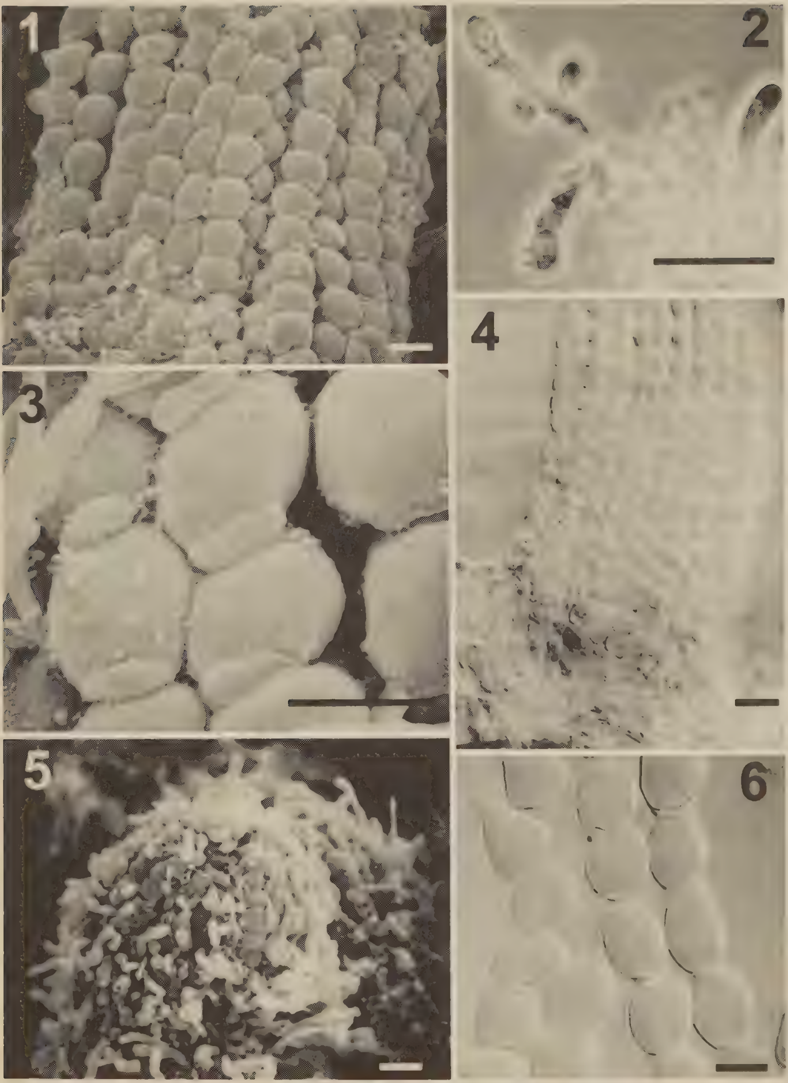
The following species of *Baeodromus* on Ranunculaceae is described as new and is the first record of a this genus on this plant family. This is the first record of *Baeodromus* from Argentina. *Baeodromus senecionis* is the only other member of this genus reported from the southern hemisphere.

***Baeodromus ranunculi*** J.R. Hern. & J.F. Hennen, *sp. nov.*

Figs 1-6.

Spermogonia, aecia et uredinia non visa. Telia amphigena, praecipue hypophylla, origine subepidermales, cerea; teliosporae unicellulares, catenulatae, valde connatae, laeves, hyalinae, globosae vel ovoideae, 15-22 x 11-15 µm, parieti cellularum minus quam 1 µm crasso, cellulis intercalaribus manifestis 2-3 x 5-6 µm.

Spermogonia, aecia, and uredinia not found. Telia amphigenous, mainly hypophyllous, subepidermal in origin, waxy (*Aecidium*-like but compact); teliospores one-celled, strongly catenulate, smooth, colorless, germ pores not observed, globoid or ovoid, 15-22 x 11-15



Figs. 1- 6. ***Baeodromus ranunculi***. 1. Telium with catenulate teliospores. 2. Metabasidia and basidiospores. 3. Teliospores and intercalary cells. 4. Telium. 5. Telium with probasidial cells producing metabasidia. 6. Catenulate teliospores and intercalary cells. (Figs. 1, 3, 5: Scanning electron microscopy; 2: phase contrast microscopy; 4, 6: transmitted light microscopy. Figs. 1, 2, 3, 6: Scale bar =10 µm. Figs 4, 5: Scale bar = 20 µm).

µm, cell walls evenly thickened, 1.0-1.5 µm, with conspicuous intercalary cells, 2-3 x 5-6 µm, attached to teliospores.

Holotype: ***Ranunculus flagelliformis*** Sm. (Ranunculaceae), Argentina, Catamarca, Dpto. Ambatos, El Rodeo, Lat. S. 28° 12.474', Long. W. 65° 52 445', 14 Nov 1999, *J.R. Hernández* (99-008) III (BPI 746516).
Additional material: Catamarca, Dpto. Ambatos, El Rodeo, Lat. S. 28° 12.474', Long. W. 65° 52 445', 23 Sept 1997, *J.F.Hennen & J.R. Hernández* (97-046) III.

Host and distribution: on *Ranunculus flagelliformis*, Argentina: Catamarca.

It is interesting to note that *Ranunculus flagelliformis* is semi-submerged in wet or swampy habitats. The telia are mainly present on the lower surface of leaves, facing into the water, suggesting that basidiospores infect nearby plants via dissemination by water.

The only other report of a rust on *R. flagelliformis* is *Aecidium ranunculi* Schwein. from Chile (Dietel & Neger, 1899), which was also reported from Brazil on *R. bonariensis* Poir. (Pazschke, 1896). These collections have not been examined, however, these reports appear to be based on misidentifications. *Aecidium ranunculi* is the aecial stage of *Puccinia eatoniae* Arthur (1934), which produces the uredinial and telial stages on *Sphenopholis* spp. (*Poaceae*), a grass not found in South America. *Puccinia eatoniae* is known from North America, Czechoslovakia and Sweden.

In addition to *Baeodromus ranunculi*, three other rusts are reported on *Ranunculus* in Argentina (Lindquist, 1982). *Puccinia andina* Dietel & Neger is found on *Ranunculus peduncularis* Sm. in Tierra del Fuego (also in Chile, Valdivia) and *Puccinia blyttiana* Lagerh. on *Ranunculus lancipetalus* Griseb. in La Rioja and Salta Provinces. The teliospores of *B. ranunculi* are different from those of species of *Puccinia*, which produces distinctive pedicellate, 1-septate, non-catenulate, usually brown teliospores.

*Aecidium negerianum* Dietel is reported from southern Argentina and Chile on *Ranunculus biternatus* Sm. (Argentina, Tierra del Fuego, Type of *Aecidium ushuwaiense* Speg., 1923), on *Ranunculus fuegianus* Speg. (Argentina, Tierra del Fuego), on *Ranunculus peduncularis* (Argentina, Tierra del Fuego; Chile, Volcán Copahue, type of *Aecidium negerianum* Dietel in Dietel & Neger, 1897), and on *Ranunculus* sp. (Argentina, Santa Cruz and Islas Malvinas, Lindquist, 1982). I consider *Aecidium negerianum* to be a species separate from *B. ranunculi* because the two rusts have never been found on the same host at the same time. *A. negerianum* has never been collected in the Northwest Region of Argentina, and it produces abundant spermogonia as compared to *Baeodromus ranunculi*, which produces no spermogonia. Therefore there is no evidence to suggest that they are two stages of the same rust species.

The only other rust reported on Ranunculaceae in Argentina is *Puccinia recondita*, which produces spermogonia and aecia on *Clematis* spp. (Lindquist, 1982).

The following key to species of *Baeodromus* is based on the literature.

1.Teliospores irregularly arranged in telia (on *Eupatorium*, from
Guatemala, Honduras and Mexico)..........................***B. eupatorii***
1.Teliospores more or less regularly catenulate in telia...................2
2. Teliospore walls thicker at the apex.............................3
2. Teliospore walls usually uniformly thick ........................4
3. Teliospores in chains of 6-14 (on *Senecio*, from Canada)...........
..................................................................***B. albertensis***
3. Teliospores in chains of 4-6 (on *Senecio*, from USA).................
..................................................................***B. californicus***
4.Intercalary cells usually present.................................5
4.Intercalary cells absent...........................................6
5. Teliospore walls 1.5-2.5 µm thick (on *Senecio*, from Mexico and
Guatemala)..................................................***B. holwayi***
5. Teliospore walls 1 µm thick (on *Ranunculus*, from Argentina)
..................................................................***B. ranunculi***
6. Teliospores in chains of 3-5 (on *Senecio*, from Ecuador)
..........................................................***B. senecionis***
6. Teliospores in chains of 2-8 (on *Urtica*, from China and
Russia)..................................................***B. tranzschelii***

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# DISCISEDA VERRUCOSA (GASTEROMYCETES) IN MEXICO

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## ABSTRACT

The presence of *Disciseda verrucosa* in the American continent (Mexico) is confirmed. Material previously recorded and new collections are described micro- and macroscopically, and were compared with the holotype. Photomicrographs under SEM are provided.

**Key words**: Lycoperdales, chorology, taxonomy.

## INTRODUCTION

*Disciseda* Czern. has been a genus unfrequently cited in the Mexican mycobiota. Up to the present study, eight species of *Disciseda* are known for Mexico (Pérez-Silva *et al.,* 1994; Esqueda-Valle *et al.,* 1995, 1998). These species are mostly from the North and Northwest Mexico and have been mainly collected from arid and semi-arid areas. Sonora is the Mexican state where this genus is best known with six taxa. Among them, *D. cervina* (Berk.) Hollós and *D. verrucosa* G. Cunn. are only known from Sonora. Aparicio-Navarro *et al.* (1994) recorded *D. verrucosa* for the first time for Mexico in Sonoran desert. They collected it during 1990. Three years later, spring of 1993, we found it in the same locality. It seems restricted to this area but samplings conducted by some of the authors during four years across the four seasons on a vegetational gradient let us find it in other type of vegetation like tropical thorn forest and tropical deciduous forest. Actually, *D. verrucosa* is only known from Sonora, Mexico in the American continent. To confirm its presence and distribution in America, all of previously recorded and new collections were compared with the holotype.

## MATERIAL AND METHODS

To analyze microscopical features of the gastrocarps, freehand samples of the gleba and peridium were mounted in water, lactophenol cotton blue, 5% KOH, and Melzer´s reagent. Electronic micrographs were made under a Jeol, JSM-5200 and a Zeiss DSM 950 S.E.M. Samples were treated in a Polaron E-2000 sputter-coater during 60 seconds at 1.2 Kv and 20 mA in an atmosphere of argon to obtain a gold coat of 500 Å. All colours were recorded according to Kornerup & Wanscher (1978). The material studied has been deposited in the National Herbarium of the Instituto de Biologia, UNAM (MEXU) and the mushroom collection of the "Centro de Estudios Superiores del Estado de Sonora" (CESUES). The localities were characterized according to biotic and abiotic factors from climatic, geologic and hydrologic charts, as well as data obtained *in situ* from soil and vegetation analyses.

## DESCRIPTION

**Disciseda verrucosa** G.Cunn. *Transactions of the New Zealand Institute 57*: 205, fig. 26, pl. XXXV, 1926. (Figs. 1-4).

Specimens examined. NEW ZEALAND, Canterbury, Waikare, *leg.* G.H. Cunningham (01.1926) Cunningham 8593, HOLOTYPE. MEXICO, Sonora, Municipio Alamos, Las Uvalamas, 26°57’43”N 108°55’30”W, *leg.* E. Pérez-Silva, M. Esqueda & I. Buendía (12.09.1994) CESUES 1778, MEXU 24086. Municipio Hermosillo, Centro Ecológico de Sonora, *leg.* A. Búrquez & A. Quijada (22.09.1990) MEXU 23582; (14.11.1990) MEXU 23587; *leg.* M. Esqueda & R.E. Villegas (07.05.1993) CESUES 1581. Municipio La Colorada, Km 100 Hermosillo–Yécora road, 28°37’11”N 110°07’09”W, *leg.* A. Armenta, A. Núñez & R. Santos (02.06.1996) CESUES 2777. Municipio San Javier, Km 137.5 Hermosillo-Yécora road, 28°34’34”N 109°46’42”W, *leg.* M. Esqueda, A. Armenta, A. Núñez & R. Santos (02.07.1997) CESUES 3295. Municipio Soyopa, Km 162.5 Hermosillo-Yécora road, 28°33’45”N 109°35’58”W, *leg.* M. Esqueda, A. Armenta, A. Núñez & R. Santos (02.07.1997) CESUES 3266.

**Fruitbody** 10-15 mm diam., depressed-globose, (Fig. 1). **Exoperidium** composed of hyphae, vegetable debris and sand grains, persisting only as a cup like sand case at the basal portion. **Endoperidium** thin, flaccid, papyraceous, violet brown (10E7) when fresh, soon umber, grayish brown (11E7) when dry, dehiscing by an inconspicuously mammose laciniate and toothed stoma about 1 mm diam. **Gleba** dark brown, powdery, whithout a sterile basal portion.

**Exoperidium** formed by hyaline, thin walled, scantily branched and septate, imperforate, quite collapsed hyphae. **Endoperidium** formed by two strata: the outermost formed by rather straight, 4-6 µm diam., thick walled and visible lumen, or solid, non-pitted hyphae; the innermost is formed by brown, thin walled, frequently septate, 3-4 µm diam., imperforate, sinuate rather straight, and loosely arranged hyphae. **Spores** (8-)8.5-12.5 x 8.5-12 µm (8.5-10 µm diam. in the holotype; Fig. 4), globoses to subgloboses, exosporium covered by small warts, and finger-like processes, most of these round and curved at the top, up to 2 µm long and also warty, specially at the base, with a stump of pedicel (Figs. 2-3). **Capillitium** 1-4 µm wide, threads light brown, flat or cylindrical, smooth or floccose, flexuous, septate, pitted.

*Disciseda* has straight to wavy capillitium, feature shared only with *Abstoma* G. Cunn., from which it differs by its typical manner of dehiscence, through a definite stoma, both characteristics are well shown in the material studied. The distinctive characters of *D. verrucosa* are the bay brown to violet endoperidium, and the globose to subglobose spores which are ornamented by finger-like processes. The specimens of Mexico are not different micro- and macroscopically from the holotype (Fig. 4). The localities of the Sonoran collections are at 200 to 1100 m height, with vegetation changing from that of the desert to tropical deciduous forest. The gastrocarps were collected under *Ceiba, Cercidium, Olneya,* and *Prosopis*. The soils had a 65-88% of sand; a pH of 6.0 to 7.6, and a total organic matter percentage of 4-6%. The contents of $NO_3^-$ and $PO_4^{=}$ were 4-10, and 6-20 mg/kg, respectively.

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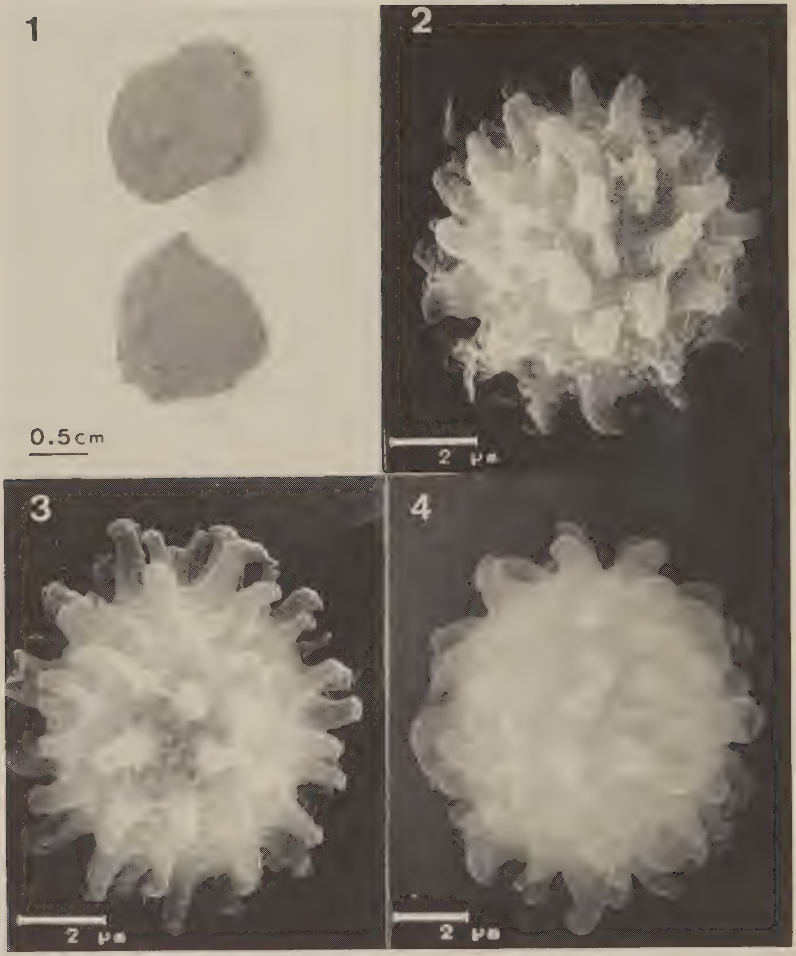

Figs. 1-4. ***Disciseda verrucosa***. Fig. 1. Fruitbodies. Figs. 2-3. Basidiospores under SEM, CESUES 1778. Fig. 4. Basidiospore under SEM, Herb.Cunningham 8593 (PDD, Holotype).

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MYCOTAXON

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# TWO CORRECTIONS TO THE CORNELL GENEALOGY OF MYCOLOGISTS

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## ABSTRACT

In a published genealogy of Cornell mycologists, F. A. Wolf was erroneously reported as H. H. Whetzel's student instead of G. F. Atkinson's student, and the legend of the photograph including both A. H. Chivers and J. H. Miller had these names inverted.

KEYWORDS: Cornell University, F. A. Wolf, history of mycology

Recently my good friend, Meredith Blackwell of Louisiana State University, brought to my attention an error in the genealogy I have published (Korf, 1991) of the mycologists in the Departments of Botany and of Plant Pathology at Cornell University. The mistake involves the position of Frederick Adolph Wolf in the genealogy, originally brought to her attention by Professor E. S. Luttrell, Wolf's student. At this time I also point out an inversion in the legend of one figure, where the order of the names fails to match the photograph.

By error I had placed Wolf and his lineage as direct "student descendents" of H. H. Whetzel of the Department of Plant Pathology. He was, however, G. F. Atkinson's Ph. D. student. That lineage should be removed from the portion of the tree called Whetzel & Generations V—VII (loc. cit., pg. 128) and F. A. Wolf should be attached instead to the fourth generation, Atkinson's students, on page. 126, *i.e.,* directly to the right of H. H. Whetzel.

F. A. Wolf's Ph. D. thesis consisted of five published papers, appearing as "Contributions of the Department of Botany, Cornell University," No. 143 (Wolf, 1912a), 144 (Wolf, 1912d), 145 (Wolf, 1911), 146 (Wolf, 1912c), and 147 (Wolf, 1912 b). In the first of these he acknowledged that "this investigation was undertaken at the suggestion of and under the careful direction of Professor George F. Atkinson, Cornell University, to whom I am grateful for help and criticism."

The other unfortunate error in my 1991 paper involves the legend for figure 15 (page 116), where the order should be "J. H. Miller, A. H. Chivers, and S. C. Teng."

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WOLF, F. A. 1912c. The brown leaf spot of colt's foot, *Tussilago farfara* L. *Ann. Mycol.* **10:** 65-67.

WOLF, F. A. 1912d. Some fungous diseases of the prickly pear, *Opuntia lindheimeri* Engelm. *Ann. Mycol.* **10:** 113-134, pl. 1-3.

MYCOTAXON

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# SCLEROSTAGONOSPORA SALSOLAE, COMB. NOV.

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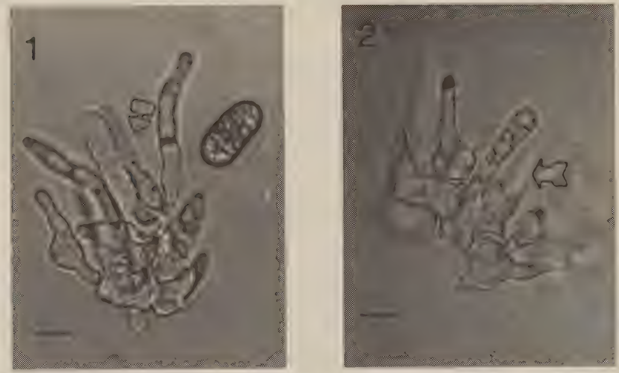
**Abstract.** A new combination *Sclerostagonospora salsolae* is proposed for the pathogenic fungus, *Hendersonia salsolae*, that causes death of *Salsola kali* ssp. *ruthenica* seedlings in Hungary.

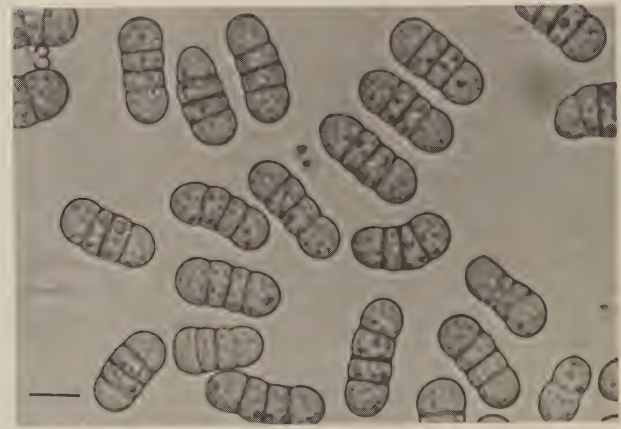
**Key Words**: *Sclerostagonospora salsolae*, morphology, *Salsola* spp., *Hendersonia salsolae,*

During a field survey at Szarkás, Hungary in June, 1996, we observed many dying seedlings of *Salsola kali* ssp. *ruthenica* (Iljin) Soó known as trumbleweed Russian thistle. The causal agent was identified as *Hendersonia salsolae* Moesz. This fungus was first described by Moesz (1926) from leaves of *Salsola kali* ssp. *ruthenica.* Morphology of the original specimen of *H. salsolae,* deposited by Moesz, was compared with our isolate and the identity was confirmed.

The generic name *Hendersonia* Berk. was formally rejected in favour of *Stagonospora* (Sacc.) Sacc. by the International Botanical Congress (IBC) in 1950 (International Code of Botanical Nomenclature, 1952). This means that the name *Hendersonia* is not available for use and that species of *Hendersonia* need to be (and largely have been) transferred to *Stagonospora.* The literature concerning rejection of the genus *Hendersonia* goes back to proposals by several mycologists (Saccardo, 1884; Maire, 1935; Wakefield, 1939). They suggested revision of *Hendersonia* because this genus involved species with different morphological characteristics, particularly in pigmentation of conidia. However, these proposals were not taken up. The proposal of the Nomenclature Committee of the British Mycological Society (Wakefield, 1939) was modified later by Rogers (1949) and accepted by the IBC in 1950. The generic name *Hendersonia* Berk. having hyaline conidia was formally rejected in favour of *Stagonospora* (Sacc.) Sacc. with *Hendersonia paludosa* Sacc. & Speg. designated as a lectotype.

Sutton (1980) proposed *Sclerostagonospora* Höhn. as a possible "recipient of the nomen rejiciendum *Hendersonia*". *Sclerostagonospora* is characterized by pale brown conidia that are 3 euseptate, pycnidial conidiomata, and holoblastic conidiogenous cells. Because the genus *Hendersonia* is a nomen rejiciendum and because morphology and conidiogenesis of our fungus, *Hendersonia salsolae*, is congeneric with the description of *Sclerostagonospora* Höhn. (Sutton, 1980), we herein transfer this species to *Sclerostagonospora*.





Figs. 1-2. Conidiogenous cells and holoblastic development of conidia of *Sclerostagonospora salsolae*. Scale bar 10µm

Fig. 3. Conidia of *Sclerostagonospora salsolae*. Scale bar 10µm

### *SCLEROSTAGONOSPORA SALSOLAE* (MOESZ) SCHWARCZINGER, VAJNA & BRUCKART, COMB. NOV.;

**Basionym:** ***Hendersonia salsolae*** Moesz, Mikológiai Közlemények VII. N° 81. in Botanikai Közlemények 23, 122. 1926. Kir. Magyar Egyetemi Nyomda, Budapest. **Holotype:** Hungary, Szigetszentmiklós, in fol. *Salsola kali* L., Aug. 31, 1925, G. Moesz. (Deposited: at the Department of Botany of the Hungarian Natural History Museum, Budapest, 1926, under the N° BP 14422).

**Symptoms** characterized by 0.5-4.0 mm diam., elongated, brown, necrotic spots on stems and leaves. Leaf spots surrounded with chlorotic or sometimes red halos. **Pycnidia** observed on dead stems and leaves. Pycnidia subglobose to globose, unilocular, immersed, separate, with single, central, papillate ostiole; dark brown at the base and light brown at ostiole; surface lacking ornamentation; walls thin, characterized as '*textura angularis*', 55-225 µm in diam. and 88-150 µm in high. Innermost layer of the pycnidial wall hyaline and bearing conidiogenous cells. **Conidiophores** absent. **Conidiogenous cells** holoblastic, discrete, determinate, ampulliform to irregular, hyaline, up to 15 µm long and 7 µm wide (Fig.1-2.). **Conidia** 8-12 X 15-25 µm (average: 10 X 21 µm), cylindrical or slightly curved, pale brown, thin-walled, rounded at both base and apex, euseptate, with 3 (rarely 1 to 4) septa. Conidia slightly constricted at the septa with occasional, rare longitudinal septa (Fig.3.).

**Specimen examined**: Hungary, Szarkás, June 1996, Schwarczinger & Vajna, on *Salsola kali* ssp. *ruthenica* stems and leaves. Deposited: at the Department of Botany of the Hungarian Natural History Museum, Budapest, N° BP 91057.

## ACKNOWLEDGEMENTS

The authors wish to thank Dr Ágnes Révay and Dr János Gönczöl from the Department of Botany of the Hungarian Natural History Museum for making the examination of the authentic material available to us. We are grateful to Dr Amy Rossman, Director of the U.S. National Fungus Collections, Beltsville, Maryland USA, for reviewing the manuscript of this paper and for her helpful comments. We thank Dr John David, Identification Service & Herbarium Manager of the CABI Bioscience for his helpful information and suggestions, as well as the Soros Fundation for the financial support of the investigations.

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# STUDIES IN NEOTROPICAL POLYPORES 8

## Poroid fungi from Jamaica - a preliminary check list

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## ABSTRACT

A checklist of 119 species of polypores of Jamaica is provided including 42 species previously not reported for the island. *Trametes tyromycoides* Ryvarden is described as new and a key to neotropical species of the genus is also provided.

Key words: Polyporaceae, Ganodermataceae, Hymenochaetaceae, Greater Antilles, West Indies, Jamaica, *Trametes*.

## INTRODUCTION

The first to report on polypores of Jamaica was Swartz (1788) who described a small number of new species based on his own collections from the island. All his species were included by Fries (1821) in his Systema mycologicum and according to Botanical Code their author citation will be Sw.:Fr.

110 years passed before Hennings (1898) published on a small collection with three species of polypores made in 1893 by Lindau. Murrill (1910) published an extensive list of 66 polypores based on collections made by several botanists from New York Botanical Garden besides his own collecting all over the island. Later he added some scattered notes on 8 Jamaican polypores (Murrill 1915, 1920, 1921), but since then there have been no reports of polypores from the island.

In 1999 a larger mycological expedition was arranged to collect macrofungi in Jamaica as a part of the Basidiomycetes of the Greater Antilles project. The expedition was split in two parts. One in which I participated, collected in the western part of the island, while the other group collected mainly around the Blue Mountains in the eastern part.

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* For previous papers in this series, see under References.

Our expedition in 1999 added 42 species to the 77 previously known from Jamaica. The large number of species that are known from only a single collection, however, suggests that our knowledge of Jamaican polypores is still incomplete and there are likely to be species that have not yet been recorded.

My own collections are deposited in the Institute of Jamaica (IJ) with some duplicates in Oslo (O). In the list they are cited with a collecting number, and further information can be extracted from the following list of localities:
Portland Ridge 5 June Coll. 41537 - 41562,
Sutton Farm, Manchester parish 8 June Coll. 41565 - 41584,
Crowlands, Trelawny parish 10 June, Coll. 41587 - 41622,
Windsor Cave, Trelawny parish 12-13 June 1999, coll. 41624 - 41691,
Reach Village, Portland parish 16 June 1999, coll. 41692 - 41711,
Hollywell nat. park, St. Andrews parish,17 June 1999, coll. 41718 - 41735.

In addition there are 7 collections made by Dr. K. Nakasone during the same expedition in the Blue Mountains. Her specimens are deposited in IJ and CFMR.

In the list, each species has a reference to the first author who reported it from the island. Those with only my or Nakasone´s collection numbers are new to Jamaica.

## Poroid fungi known from Jamaica

**CORTICIACEAE** (Poroid representatives):
**Grammothele** Berk & Curt.,
*G. fuligo* (Berk. & Br.) Ryvarden 41702.
**Porogramme** Pat.,
*P. albocincta* (Cooke & Masse) Lowe, 41631.
**Trechispora** P. Karsten,
*T. regularis* (Murrill) Liberta, Murrill 1920:87.

**GANODERMATACEAE**
**Ganoderma** P. Karsten,
*G. australe* (Fr.) Pat. , 41193.
*G. oerstedtii* (Fr.) Torrend, Murrill 1910:194.
*G. resinaceum* Boud., 41562.

**HYMENOCHAETACEAE**
**Cyclomyces** Fr.,

*C. iodinus* (Mont.) Pat., Murrill 1910:188.
*C. tabacinus* (Mont.) Pat., 41676.
**Inonotus** P. Karsten,
*I. fulvomelleus* Murrill, Murrill 1910:189.
*I. jamaicensis* Murrill, Murrill 1910:189.
**Phellinus** Quel.,
*P. apiahynus* (Speg.) Rachjenb. & Wright, 41558.
*P. calcitratus* (Berk. & W.A. Curtis) Ryvarden, Murrill 1910:194.
*P. chryseus* (Lev.) Ryvarden, 41616.
*P. cinchonensis* (Murrill) Ryvarden, Murrill 1910:194.
*P. contiguus* (Fr.) Pat., 41555.
*P. dependens* (Murrill) Imazeki, Murrill 1910:195.
*P. extensus* (Lév.) Pat., Murrill 1910:195.
*P. ferrugineo-velutinus* (Henn.) Ryvarden, 41665.
*P. gilvus* (Schw.) Pat., Murrill 1910:188.
*P. grenadensis* (Murr.) Ryvarden, Nakasone 150348,
Portland par. Cinchona Garden, 12. June 1999.
*P. nilgheriensis* (Berk. & Mont.) Cunning., Murrill 1910:169, 41627.
*P. portoricensis* (Overholts.) Fidalgo 41610.
*P. punctatus* (Fr.) Pilat, Murrill 1910:184.
*P. rhytiphloeus* (Mont.) Ryvarden, Murrill 1910:196.
*P. robustus* (P. Karsten) Bourdot & Galzin, Murrill 1910:195.
*P. roseocinereus* (Murrill) Reid, Murrill 1910:196, 41725.
*P. rufitinctus* (Cooke) Pat., Murrill 1910:184.
*P. sanctigeorgii* (Pat.) Ryvarden, Murrill 1910:195.
*P. shaferi* (Murrill) Ryvarden, 41693.
*P. umbrinellus* (Bres.) Ryvarden, Murrill 1910:184.
*P. undulatus* (Murrill) Ryvarden, 41631.
*P. viticola* (Schwein.) Donk, Murrill 1910:184.
*P. wahlbergii* (Fr.) Reid, Murrill 1910:196.
**Phylloporia** Murrill,
*P. chrysita* (Berk.) Ryvarden, Murrill 1910:189.
*P. spatulatha* (Hooker) Ryvarden, Murrill 1910:185.

**POLYPORACEAE** (s. lato)
**Abortiporus** Murrill,
*A. fractipes* (Berk. & W.A. Curtis) Murrill 1910:185, Nakasone 150357, 150393, St. Andrews parish, Blue Mt. Nat. Park, Hollywell recreation area, 10 June 1999.
**Antrodia** P. Karsten,
*A. albida* (Fr.) Donk, Murrill 1910:185.

**Antrodiella** Ryvarden & Johansen,
*A. hydrophila* (Berk. & W.A. Curtis) Ryvarden, Nakasone 150349 Portland parish, Cinchona Garden, 12. June 1999.
*A. semisupina* (Berk. & W.A. Curtis) Ryvarden, Murrill 1910:191.
**Bjerkandera** P. Karsten,
*B. adusta* (Fr.) P. Karsten, Murrill 1910:185.
**Ceriporia** Donk,
*C. tarda* (Berk.) Ginns, 41552.
*C. xylostromatoides* (Berk.) Ryvarden, Murrill 1920:85.
**Ceriporiopsis** Domanski,
*C. flavilutea* (Murrill) Ryvarden, 41594.
*C. umbrinescens* (Murrill) Ryvarden, Murrill 1920:83, 41661.
**Coriolopsis** Murrill,
*C. polyzona* (Pers.) Ryvarden, Murrill 1910:186.
*C. rigida* (Berk. & Mont.) Murrill, Murrill 1910:186.
**Datronia** Donk,
*D. caperata* (Berk.) Ryvarden, Murrill 1910:186, 41587.
*D. fulvocinerea* (Murrill) Ryvarden, Murrill 1910:186.
*D. taylorii* (Murrill) Ryvarden, Murrill 1910:186.
**Dichomitus** Reid,
*D. cavernulosus* (Berk. & W.A. Curtis) Masuka & Ryvarden, 41688.
**Earliella** Murrill,
*E. scabrosa* (Pers.) Gilb. & Ryvarden, 41600.
**Echinopora** Ryvarden,
*E. aculeifera* (Berk. & W.A. Curtis) Ryvarden, 41594.
**Flaviporus** Murrill,
*F. brownii* (Humboldt) Donk, Murrill 1910:188.
**Flavodon** Ryvarden,
*F. flavus* (Kl.) Ryvarden, 41548.
**Fomes** (Fr.) Fr.,
*F. fasciatus* (Sw.:Fr.) Cooke, Swartz 1788:1922..
**Fomitella** Murrill,
*F. supina* (Sw:Fr.) Murrill, Swartz 1788:1926.
**Fomitopsis** P. Karsten,
*F. dochmia* (Berk.) Ryvarden, Murrill 1910:194, 41624.
*F. meliae* (Underw.) Gilb. & Ryvarden, Murrill 1910:195.
*F. nivosa* (Berk.) Gilb. & Ryvarden, Murrill 1910:193.
**Fuscocerrena** Ryvarden,
*F. portoricensis* (Fr.) Ryvarden, Murrill 1910:185, 41583.
**Gloeophyllum** P. Karsten,
*G. berkleyii* (Mont.) Ryvarden, Murrill 1910:197.

*G. striatum* (Swartz:Fr.) Murrill, Murrill 1910:197.
**Heterobasidion** Brefeld,
*H. annosum* (Fr.) Brefeld, Nakasone 150281,
St. Andrews parish, Blue Mt. Nat. Park, Hollywell recreation area, 10 June 1999.
**Hexagonia** Fr.,
*H. hydnoides* (Sw:Fr.) Fidalgo, Murrill 1910:190.
*H. leprosa* Fr., Murrill 1910:191.
**Junghuhnia** Corda,
*J. undigera* (Berk.) Ryvarden, Murrill 1910:187.
*J. subundata* (Murrill) Ryvarden, 41614.
**Laetiporus** Murrill,
*L. percicinus* (Berk. & W.A. Curtis) Gilb., Murrill 1910:193.
*L. sulphureus* (Fr.) Murrill, 41726.
**Megasporoporia** Ryvarden & Wright,
*M. setulosa* (Henn.) Rajchenb., 41574.
**Microporellus** Murrill,
*M. dealbatus* (Berk. & W.A. Curtis) Murrill, Murrill 1910:189.
**Nigrofomes** Murrill,
*N. melanoporus* (Mont.) Murrill, Murrill 1910:194.
**Nigroporus** Murrill,
*N. vinosus* (Berk.) Murr., Murrill 1910:190.
**Oligoporus** Bref.,
*O. caesius* (Fr.) Gilb. & Ryvarden, Murrill 1910:192.
*O. floriformis* (Quel.) Gilb. & Ryvarden Murrill 1910:192.
*O. rancidus* (Bres.) Gilb. & Ryvarden, Nakasone 150362,
St. Andrews parish, Blue Mt. Nat. park, Hollywell recreation area, 10 June 1999.
**Oxyporus** Donk,
*O. latemarginatus* (Durand & Mont.) Donk, Murrill 1920:305.
**Pachykytospora** Kotl. & Pouzar,
*P. alabamae* (Schwein.) Ryvarden, 41565.
**Perenniporia** Murrill,
*P. contraria* (Berk. & W.A. Curtis) Ryvarden, 41607.
*P. cremeopora* Decock & Ryvarden, 41730.
*P. inflexibilis* (Berk.) Ryvarden, Murrill 1910:196.
*P. medullapanis* (Fr.) Donk, 41545.
*P. ochroleuca* (Berk.) Ryvarden, Murrill 1910:194.
*P. tephropora* (Mont.) Ryvarden, Murrill 1910:184.
**Polyporus** Fr.,
*P. arcularius* Fr., Murrill 1910:190.

*P. dictyopus* Mont., Murrill 1910:190.
*P. leprieurii* Mont., Murrill 1910:190.
*P. tenuiculus* Beauv.:Fr., Murrill 1910:190.
*P. tricholoma* Mont., 41580.
**Protomerulius** Møller,
*P. substuppeus* (Berk. & W.A. Curtis) Ryvarden, 41683.
**Pseudofavolus** Pat.,
*P. cucullatus* (Mont.) Pat., Murrill 1910:189.
**Pycnoporus** Murrill,
*P. sanguineus* (L: Fr.) Murrill, 41689.
**Rigidoporus** Murrill,
*R. lineatus* (Pers.) Ryvarden, 41575.
*R. microporus* (Sw:Fr.) Overeem, Swartz 1788:1925.
*R. ulmarius* (Fr.) Imazeki, Murrill 1910:194.
*R. undatus* (DC) Donk, 41733.
*R. vinctus* (Berk.) Ryvarden, 41722.
**Schizopora** Vel.,
*S. flavipora* (Cooke) Ryvarden, 41670.
**Skeletocutis** Keller,
*S. lenis* (P. Karsten) Niem., Murrill 1920:90, 41673.
*S. nivea* (Jungh.) Keller, Nakasone 150277, St. Andrews parish, Blue Mt. Nat. Park, Hollywell recreation area, 10 June 1999.
*S. niveicolor* (Murrill) Ryvarden, Murrill 1920:87.
**Tinctoporellus** Ryvarden,
*T. epimiltinus* (Berk.) Ryvarden, Murrill 1920:87, 41566.
**Trametes** Fr.,
*T. cubensis* (Mont.) Pat. & Lagerh., Murrill 1910:191, 41682.
*T. elegans* (Spreng.:Fr.) Fr., Hennings 1898:281, Murrill 1910:196.
*T. lactinea* Berk., 41617.
*T. maxima* (Mont.) David & Rajchenb., Murrill 1910:187, 41625.
*T. membranacea* (Sw.:Fr.) Kreisel, Swartz 1788:1922.
*T. pavonia* (Hook.) Ryvarden, Hennings 1898:280, Murrill 1910:187.
*T. versicolor* (Fr.) Pilat, Murrill 1910:188.
*T. villosa* (Sw.:Fr.) Kreisel, Swartz 1788:1923.
**Trichaptum** Murrill,
*T. perrottetii* (Lev.) Ryvarden, Murrill 1910:186, 41675.
*T. sector* (Ehrenb.:Fr.) Kreisel, Murrill 1910:188.
**Tyromyces** P. Karsten,
*T. leucomallus* (Berk. & W.A. Curtis) Murrill, Murrill 1910:192.
*T. preguttulatus* (Murrill) Ryvarden, Murrill 1910:190.
**Wrightoporia** Pouzar,

*W. avellanea* (Bres.) Pouzar, Murrill 1920:92, 41710
*W. bracei* (Murrill) Ryvarden, 41620.
*W. lenta* (Overholts & Lowe) Pouzar, Nakasone 150311,
Portland par. Cinchona Garden, 12. June 1999.
*W. micropora* Ryvarden 41606.
*W. tropicalis* (Cooke) Ryvarden, 41692.

**Trametes tyromycoides** Ryvarden nov. sp. Fig. 1 A-D
Fructificatio sessilis, pileus ochraceus, pori rotundis vel angulatis, 5-8 per mm, brunneus, systema hyphale dimiticum, hyphae generatoriae, fibulatae, hyphae skeletales hyalinae, basidiosporae cylindricae, 6.5-7.5 x 2.8-3.2 µm.
Holotypus: Jamaica, Trelawny parish, Windsor Caves, on dead hardwood log, 12 June 1999, L. Ryvarden 41691, holotype in IJ, isotype in O & CFMR
**Basidiocarps** pileate, broadly sessile, partly imbricate, individual pilei applanate, shelf-like and tough when fresh, slightly contracted, deflexed and dense when dry, up to 1.5 cm thick at the base and 5 cm wide and long, upper surface glabrous, slightly tuberculate and partly radially lined, first whitish, later more discoloured in cream and pale brown colours, pore surface whitish when fresh, resinous brown when dry, pores thin-walled, angular to wavy, but entire, 5-8 per mm, tube layer concolorous with pores, up to 10 mm thick, context whitish becoming ochraceous and strongly contrasting the resinous brown tubes.
**Hyphal system** dimitic, generative hyphae hyaline, with clamps, 3-5 µm wide, skeletal hyphae scattered in the trama, predominant in the context, solid to thick-walled, hyaline, mostly unbranched, occasionally with a dichotomous branching and some as if twisted, negative in Melzer's reagent, 3-8 µm in diam.
**Cystidia** and other sterile hymenial elements absent.
**Basidia** 15-20 x 5-6 µm with basal clamps and 4 sterigmata.
**Basidiospores** cylindrical, thin-walled, negative in Melzer's reagent, 6.5-7.5 x 2.8-3.2 µm.
**Type of rot.** White rot.
**Substrate**. On dead hardwood log.

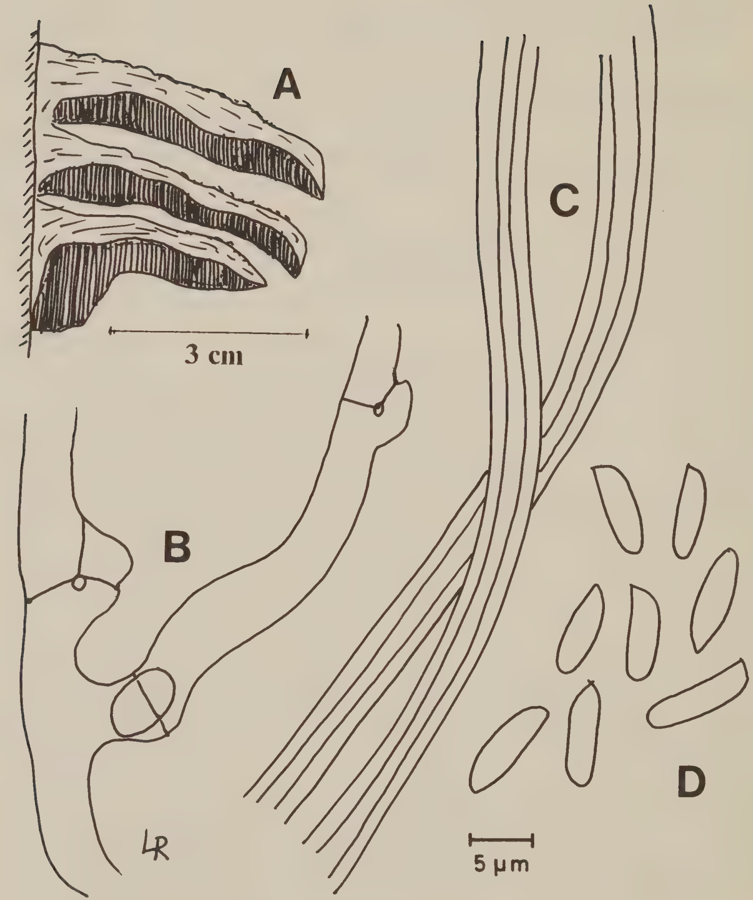

Fig. 1 *Trametes tyromycoides* A) Section of the holotype, B) Generative hyphae, C) Skeletal hyphae, D) Basidiospores. From the holotype.

**Distribution.** Known only from the type locality.
**Remarks.** The type collection was taken for a *Tyromyces* species in the field because of its fleshy and soft consistency when fresh, a slight contraction and change of tube colour during the drying. In this respect it is reminiscent of *T. fisslis* (Berk. & W.A. Curtis) Donk.

However, a microscopical examination demonstrated that the hyphal construction was dimitic with skeletal hyphae more or less dominating in the context where only few generative hyphae could be observed. The cylindrical basidiospores are of a type commonly seen in *Trametes* and this and the dimitic hyphal system made it more natural to describe the new species in this genus rather in *Tyromyces.* A key to known neotropical *Trametes* species is given below.

**Key to neotropical *Trametes* species**
1. Pores regular, 1-3 per mm or larger, lamellate, daedaleoid, semilabyrinthine or lacerate to almost hydnoid........................................2
1. Pores regular, 3-8 per mm, round to angular, more or less entire .........6

2. Upper surface more or less glabrous................................................ 3
2. Upper surface hirsute to hispid ........................................................4

3. Hymenophore often lamellate or pores sinuous to daedaleoid in parts, cystidia absent, common species ............................................**T. elegans**
3. Pores angular 1-4 mm wide, finely encrusted cystidia present ..................................................................................................**T. cystidiata**

4. Basidiocarp thin and flexible, rarely above 3 mm thick ........ **T. villosa**
4. Basidiocarp hard and rigid, up to 1.5 cm thick ................................ 5

5. Context duplex with a distinct black zone, at least close to the base; hymenophore split and almost hydnoid, spores 4.5-5.5 µm long ..................................................................................................**T. maxima**
5. Basidiocarp up to 2 cm wide, often effused reflexed, homogenous to duplex, but without a black zone; hymenophore regular, to slightly daedaleoid, about 1 mm wide, spores 7-9 µm long..................... **T. cervina**

6. Pileus hirsute to tomentose; context duplex, often with a black line between tomentum and context, at least close to the base .........................7
6. Pileus adpressed velutinate and dull to sub shiny or soon becoming glabrous except for margin; context homogeneous although a cuticle may

develop from the base with age............................................................ 9

7. Pileus usually strongly multizonate, often in different colours as alternating tomentose and glabrous zones  pore surface white becoming pale tan with age .................................................. **T. versicolor**
7. Pileus azonate or with zones in different colours of white to ochraceous never a black zone below the tomentum ..................................7

8. Pileus tomentose in zones, white to buff or pale brown; pores angular, often slightly elongated radially; spores ellipsoid, 5-6 x 3-4 µm ..........................................................................................**T. pavonia**
8. Pileus finely adpressed velutinate, becoming almost glabrous with age, white, pale tan or pale cinnamon; pores round to regular; spores cylindric, narrower than above, spores 4.5-6 x 2-2.5 µm ................ **T. membranacea**

9. Pores 1-3 per mm ..........................................................................10
9. Pores 4-7 per mm ..........................................................................11

10. Spores 10-15 µm long, skeletal hyphae dextrinoid ............ **T. frustrata**
10. Spores 4-7 µm long, skeletal hyphae non dextrinoid............ **T. lactinea**

11. Dark reddish, brown or blackish cuticle spreading from the base .....12
11. No cuticle spreading from the base, upper surface white, ochraceous becoming unevenly pale brown with age .............................................. 14

12. Upper surface becoming greyish and black from base ...... **T. cingulata**
12. Upper surface becoming tan, brown to reddish from base or in zones ...........................................................................................................13

13. Upper surface usually zonate with variable colours in brown shades, no pointed hyphal ends in the hymenium .................................. **T. ectypus**
13. Upper surface azonate, becoming reddish from the base, sharply pointed hyphal ends in the hymenium ..................................... **T. cubensis**

14. Context pale pinkish to cafe au lait, red to brownish with KOH but fading to dark spot ..........................................................................15
14. Context white to ochraceous or cork coloured ..................................16

15. Upper surface soft velvety to glabrous in zones, spores 1.5-2 µm wide ......................................................................................... **T. modesta**

15. Upper surface glabrous, spores 2.5-3 µm wide............................ **T. roseola**

16. Spores cylindrical.................................................................17
16. Spores ellipsoid ..................................................................19

17. Pores 5-8 per mm, pore layer resinous brown when dry, context whitish ...................................................................... **T. tyromycoides**
17 Pores 3- 4 per mm, pore layer and context concolorous when dry ....18

18. Basidiocarp effused reflexed, pileus flexible and papery thin, spores 7-10 µm long............................................................. **T. cotonea**
18. Basidiocarp sessile to dimidiate, tough, up to 6 mm thick, spores 6-7 µm long........................................................................ **T. marianna**

19. Pileus surface even, spores 3-4 x 2.5-3 µm ................. **T. ellipsospora**
19. Pileus surface uneven, tuberculate to crested, spores not known ........................................................................................**T. ochroflava**

**ACKNOWLEDGEMENTS**
The field work in Jamaica was funded under the US National Science Foundation Biotic Surveys Inventories Programme, BS NSF DEB-9525902 with additional support from the International Institute of Tropical Forestry. Dr. D. J Lodge of USDA-FS, FPL in Puerto Rico and Dr. T. J. Baroni of State University New York, Cortland, and Tracey Commock of the Institute of Jamaica organized the expedition and took care of all practical details and their enthusiasm and support are deeply acknowledged. Dr. D. J. Lodge has kindly acted as presubmission reviewer and has suggested improvements in the manuscript. Dr. K. Nakasone kindly left me her Jamaican polypores for incorporation in the list and her generosity is appreciated.

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MYCOTAXON

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# FIRST REPORT OF PERSICIOSPORA MOREAUI, A PARASITE OF FUSARIUM OXYSPORUM, IN THE WESTERN HEMISPHERE

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## ABSTRACT

A pyrenomycetous ascomycete was isolated from infected watermelon roots in association with the Fusarium wilt pathogen (*Fusarium oxysporum* f. sp. *niveum*). Single spored cultures produced ostiolate perithecia measuring 250-300 μm in diameter, with dark, ellipsoid ascospores (20-22 x 8-11 μm) each containing two apical germ pores. The surface of the ascospores was finely pitted with no additional ornamentation. Cultures additionally produced a creamy tan to straw-colored mycelium with a *Phialophora*-like conidial state. Based upon these characteristics, the fungus was identified as *Persiciospora moreaui*. It was demonstrated to be parasitic on the watermelon wilt pathogen but caused no appreciable damage in vitro. The results of this study represent a new report for this fungus in the Western Hemisphere, and also provides the first conclusive evidence that it is capable of functioning as a mycoparasite.

**Keywords:** Pyrenomycetes, Ascomycetes, mycoparasites

## INTRODUCTION

*Persiciospora moreaui* (Doguet) Cannon & Hawksw., was first described by Doguet (1955) as *Melanospora moreaui*. However, the taxon was invalidly published because no Latin diagnosis was included. Von Arx (1981) later transferred the species to *Sphaerodes*. Cannon and Hawksworth (1982) revised the genus *Melanospora* and separated it from related genera based on shape and ornamentation of ascospores. Because spore ornamentation of this fungus was distinct from that of *Melanospora* and *Sphaerodes*, Cannon and Hawksworth (1982) described two species, *P. moreaui* and *P. masonii*, and transferred them to the new genus *Persiciospora*. Two additional species have subsequently been described, *P. japonica* from Japan (Horie et al. 1986), and *P. africana* from South Africa (Krug, 1988). The derivation of this name stems from the Latin "Persicum" for peach, which refers to the similarity of the ascospores to a peach seed.

## Identification

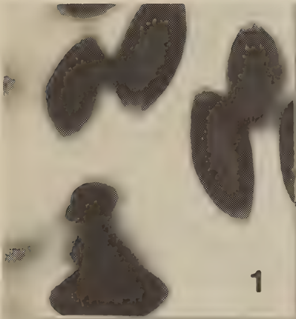
*Persiciospora moreaui* has been characterized by ostiolate, pale to golden yellow, spherical perithecia 250-350 μm in diameter, with a neck 20-100 μm long ringed with hyaline setae (Cannon and Hawksworth, 1982). It also has deliquescent, clavate, 8-spored asci measuring 65-75 x 20-25 μm. Ascospores (20-24 x 8-11 μm) are dark, ellipsoid, and pitted like a peach seed (Fig. 1). They additionally have two apical germ pores (Figs. 2,3).

Cannon and Hawksworth (1982) state that *P. moreaui* has been found only twice. The first was credited to Moreau, and reported by Doguet (1955). However, no location was given for its finding, but was assumed to be from France (Cannon and Hawksworth, 1982). The other location was from New Zealand and has been designated as the holotype (Cannon and Hawksworth, 1982). Both reports describe isolation of the fungus in conjunction with *F. oxysporum*, but parasitism was only assumed, but not investigated experimentally. This brief report describes the identification of *P. moreaui* from a new geographic location and demonstrates the parasitic relationship of the fungus with *F. oxysporum* at the bench level.

## Isolation

The fungus was found growing in dual culture with *F. oxysporum* f. sp. *niveum* (FON) from a diseased field specimen collected from Gilchrist Co., Florida. Watermelon root pieces presumed to be infected with the wilt pathogen were surface sterilized and plated on acidified potato dextrose agar (APDA). After 2-3 wks, dark perithecia were observed in plates intermingled with the white *Fusarium* hyphae. Single-spored cultures were obtained by picking perithecia from the plates and shaking in 2 ml water blanks amended with 50% lactic acid. After several minutes, the water was overlayed on PDA plates and incubated overnight at room temperature. The following day, individual ascospores were removed with the aid of a dissecting microscope and transferred to PDA or Mycological agar amended with 100 ml/l of a filtrate from a 3-4 day old liquid culture of FON. These plates were incubated at 25°C. It had previously been discovered that the fungus grew and sporulated more efficiently after being grown on media with an added filtrate of the host (Harveson and Kimbrough, 1998).

Figs 1-7. Culture and morphology of *Persiciospora moreaui.* **Fig. 1.** Darkly pigmented, pitted ascospores (X1500). **Fig. 2.** Germination from the polar pores (X350). **Fig. 3.** Apical germ pore (arrow) (X1500). **Fig. 4.** A mature perithecium with hyaline peridium, and ascospores exuding through ostiole (X150). **Fig. 5.** Young asci before deliquesance (X440). **Fig. 6.** Hyphal strand with several phialidic-type anamorphs (X365). **Fig. 7.** PDA/Komada culture plate showing the dark growth of *P. moreaui*, (arrows) lighter growth of *Fusarium oxysporium* f. sp. *niveum*, and a central darker zone of *P. moreaui* perithecia (X0.8).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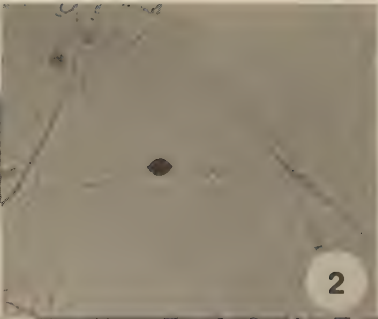
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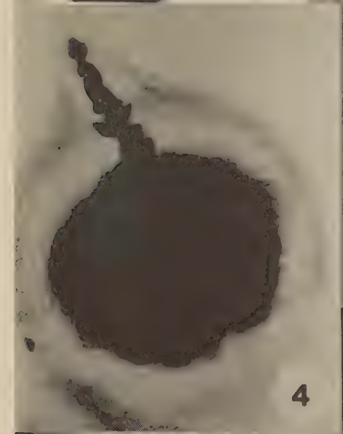
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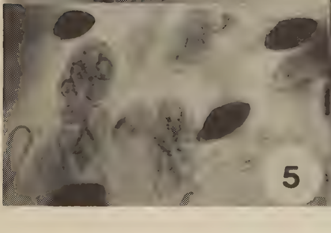
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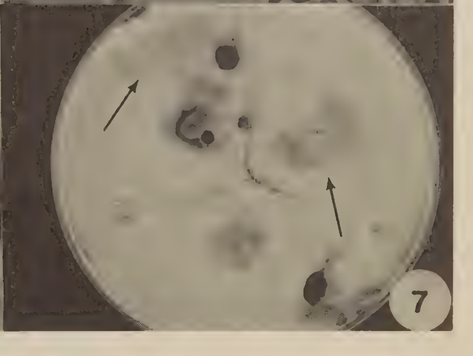
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Germinating ascospores produced a tan to straw-colored mycelium on PDA. Perithecia were ostiolate, 270-280 μm in diameter, with spores measuring 20-22 x 8-10 μm, but no setae were observed on the perithecial neck as was described for the holotype (Cannon and Hawksworth, 1982), (Fig. 4). Perithecia also tended to be gregarious in culture, and their amber color appeared dark to the naked eye when filled with mature ascospores. Ascospores were ellipsoid and smooth in outline, with the distinctive pitted or pore-like surface typical of the genus (Fig. 1). Asci from this specimen, however, were smaller than those reported for the holotype, measuring 20-22 x 40-45 μm (Fig. 5). This fungus additionally produced a *Phialophora*-like anamorph (Fig. 6) that was indistinguishable from the allied genera *Melanospora* and *Sphaerodes* (Harveson and Kimbrough, 1998). Based upon these characteristics, and following Cannon and Hawksworth's taxonomic revision (Cannon and Hawksworth, 1982), the fungus was identified as *Persiciospora moreaui*.

## Proof of Parasitism

Parasitism was observed by slide cultures of *P. moreaui* and FON. Germinating ascospores formed small knobs or contact branches like those described by Jordan and Barnett (1978) from *Melanospora zamiae*. This served as their criterion for evaluating parasitism. An additional assay was developed to provide further evidence of parasitism. It consisted of standard 100 x 15 mm petri plates and two different media. A sterile 40 mm diameter beaker was placed in the middle of a plate and molten PDA was poured around the outside. After the agar solidified, the beaker was removed, leaving a 40 mm diameter hole in the middle ringed by PDA. The hole was gently filled with Komada's medium, the selective medium for *F. oxysporum* (Komada, 1975). Previous work had shown that *Persiciospora* was completely inhibited on this growth medium (Harveson and Kimbrough, 1998). Plugs of actively growing cultures of both *P. moreaui* and FON (8 mm) were placed in the center section of plates containing the Komada's medium. After 2-3 wks at 25°C, perithecia and hyphal branches covered with the anamorphic state were observed on both media (Fig. 7). The hyphae of *P. moreaui* was readily distinguished from that of FON by its tan color that contrasted well with the white of the *Fusarium* (Fig.7). Because of *Persiciospora*'s inability to grow on Komada, it was concluded that the formation of new perithecia at the periphery of the plate proved that the fungus was parasitising and utilizing the *Fusarium* hyphae for nutrients as it grew out from the center. Although sporulation and hyphal growth occurred on the Komada, it was more prevalent on the outside ring of PDA, which was previously identified as a good medium for growth and perithecial production of *P. moreaui*.

## FON Inhibition

Another assay was developed to evaluate the extent of hyphal reduction or damage to FON in vitro. Eight mm agar plugs of both fungi were placed 6 cm apart on PDA and Mycological agar plates, utilizing five replications per assay. These plates were compared to FON growing singly on the same media. Linear growth of FON for both treatments was measured daily for 12 days, and the experiment was repeated once. Collected data were analyzed as a repeated measures experiment and additionally subjected to linear regression using the quadratic model: $y = a(trt) + b(obs) + c(obs^2)$

where y = growth response and a, b, and c were parameters to be estimated. Since no significant differences existed between repeated tests or the two media evaluated, all observations were combined to include 12 data points from each of 20 replications. The results are illustrated in Table 1, and show that no significant differences existed between the hyphal growth of FON alone and that produced in dual culture with both fungi. This indicates that growth inhibition caused by the parasitism of FON by *P. moreaui* is minimal.

Table 1. Least square mean values and pairwise comparison probability values for the parasitism of *Persiciospora moreaui* on *Fusarium oxysporum* f. sp. *niveum* (FON).

| | LS Mean (mm) | Pr > ITI |
|---|---|---|
| FON | 50.8 | - |
| *Persiciospora moreaui* & FON (Dual Culture) | 49.0 | 0.0024* |

* = not significantly different after being subjected to the Bonferroni correction (P = 0.0016)

## Commentary

The significance of this report stems from the fact that it describes the existence of a rarely found, soil-inhabiting ascomycete from a new geographic region of the world. To our knowledge, this represents the third time it has ever been reported. It also provides conclusive evidence that the fungus is parasitic in nature on *F. oxysporum*. This observation has been assumed previously, but not proven (Cannon and Hawksworth, 1982). However, while it is true that *P. moreaui* is parasitic on *F. oxysporum*, it is apparently not pathogenic. This suggests that it functions ecologically as a mutualistic or commensualistic parasite. It was further determined that the fungus can reside saprophytically in culture, but grows and sporulates more efficiently in the presence of *Fusarium* or some metabolite produced by *Fusarium* in the form of an added filtrate.

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# STUDIES ON CERCOSPORA AND ALLIED GENERA IN CHINA VI [*]

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ABSTRACT: Two new species, *Mycovellosiella clematidis* Y. L. Guo on *Clematis* sp. and *Mycovellosiella codonopsis* Y. L. Guo on *Codonopsis* sp. are described and illustrated and the followings one new combination and four new records for China are introduced, *Pseudocercospora vaccinii* (Kats. & Kob.) Y. L. Guo, *Cercospora commelinicola* Chupp ex U. Braun, *Cercospora cosmi* Chidd., *Cercospora ratibidae* Ellis & Barthol. and *Cercospora sonchi* Chupp. Specimens examined are deposited in HMAS.

KEY WORDS: *Mycovellosiella*, *M. clematidis*, *M. codonopsis*, new records, China.

**Cercospora commelinicola** Chupp ex U. Braun, Crypt. Mycol. 20(3): 156, 1999.

On *Amischotolype hooker* (Hassk.) Hara: Xiaomenglun. Yunnan Province (HMAS 79275).

On *Commelina communis* L.: Longjin, Guangxi Province (HMAS 77694).

On *Commelina paludosa* Bl.: Wuzhishan, Hainan Province (HMAS 77695).

**Cercospora cosmi** Chidd., Sydowia 13: 157, 1959.

On *Cosmos bipinnatus* Cav.: Emeishan, Sichuan Province (HMAS 77723).

**Cercospora ratibidae** Ellis & Barthol., Journ. Mycol. 8: 177, 1902.

On *Saussurea* sp.: Dabashan, Sichuan Province (HMAS 77734).

**Cercospora sonchi** Chupp, A Monograph of the Fungus Genus *Cercospora*: 159, 1954.

On *Taraxacum* sp.: Beijing (HMAS 77735).

**Mycovellosiella clematidis** Y. L. Guo, sp. nov. Fig. 1

Maculae amphigenae, angulares vel irregulares, 1-4 mm latae, confluentes, atrobrunneae vel subnigrae. Caespituli hypophylli. Mycelium secundarium superficiale: hyphae subhyalinae, ramosae, septatae, 2-2.5 μm latae. Stromata nulla vel parvula, olivaceo-brunnea. Conidiophora laxe fasciculata vel ex hyphis myceliis secundariis oriunda, pallide olivacea, erecta vel leviter curvata, 0-2-geniculata, raro ramosa, 0-2-septata, 22-50 × 4.3-6.5 μm. Cicatrices conidiales conspicuae, 2-2.5 μm latae. Conidia solitaria, obclavata, subhyalina, erecta vel curvata, ad apicem obtusa, ad basim obconico-truncata, 3-7-septata, 30-65(-88) × 3.2-4.5 μm.

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[*] Supported by the National Natural Science Foundation of China (Approved No. 39899400)

Hab. in foliis vivis *Clematis* sp.: China, Lijiang, Yunnan Provincia, 23 IX 1987, leg. Guo YL, no.1090 (HMAS 80313, Holotypus).

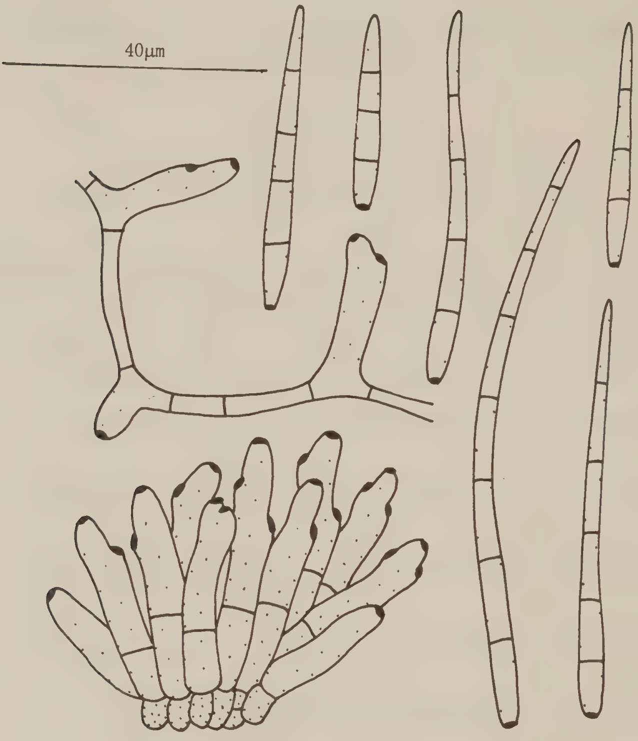

Fig.1 *Mycovellosiella clematidis* Y. L. Guo

Leaf spots amphigenous, angular to irregular, 1-4 mm wide, often confluent, deep brown to blackish, with pale olivaceous to pale yellowish brown halo on the upper surface, grayish brown on the lower surface. Fruiting hypogenous. Secondary mycelium external: hyphae emerging through stromata, subhyaline, branched, septate, 2-2.5 μm wide. Stromata none or small, substomatal, olivaceous brown. Conidiophores emerging through stomata, loosely fasciculate, pale olivaceous, uniform in colour, irregular in width, straight to slightly curved, 0-2-geniculate, rarely branched, rounded to conical at the apex, 0-2-septate, sometimes constricted, 22-50 × 4.3-6.5 μm. Conidial scars conspicuously thickened, 2-2.5 μm wide. Conidia solitary, obclavate, subhyaline, straight to curved, obtuse at the apex, obconically truncate at the base, 3-7-septate, 30-65(-88) × 3.2-4.5 μm.

*Cercospora clematidis* Boedijn (1961) is similar to the new species, but differs from

this fungus by lacking external hyphae, brown, longer, and narrower conidiophores (21-94 × 3-4 μm), and acicular, hyaline, slightly narrower conidia (48-84 × 2.5-3.5 μm). The condial scars are inconspicuous, and this species is identical with *Pseudocercospora clematidis* Goh & W. H. Hsieh.

**Mycovellosiella codonopsis** Y. L. Guo, sp. nov. Fig. 2

Maculae amphigenae, orbiculares vel irregulares, 1-5 mm diam., pallide flavo-brunneae vel griseo-brunneae. Caespituli hypophylli. Mycelium secundarium superficiale: hyphae pallide olivaceae, ramosae, septatae, 2.2-3.7 μm latae. Stromata nulla. Conidiophora 2-16 fasciculata vel ex hyphis myceliis secundariis oriunda, olivacea vel olivaceo-brunnea, erecta vel leviter curvata, geniculata, non ramosa vel ramosa, 0-4-septata, constricta, 4.3-86.5 × 4.3-8.7 μm. Cicatrices conspicuae, 2.2-3.2 μm latae. Conidia obclavata vel cylindrica, olivacea, catenata, erecta vel curvata, ad apicem rotundata vel conica, ad basim obconico-truncata, 1-7-septata, interdum constricta, 22-91 × 4.8-7.6 μm.

Hab. in foliis vivis *Codonopsis* sp.: China, Emeishan, Sichuan Provincia, 23 X 1958, leg. Song MH, no.328 (HMAS 79137, Holotypus).

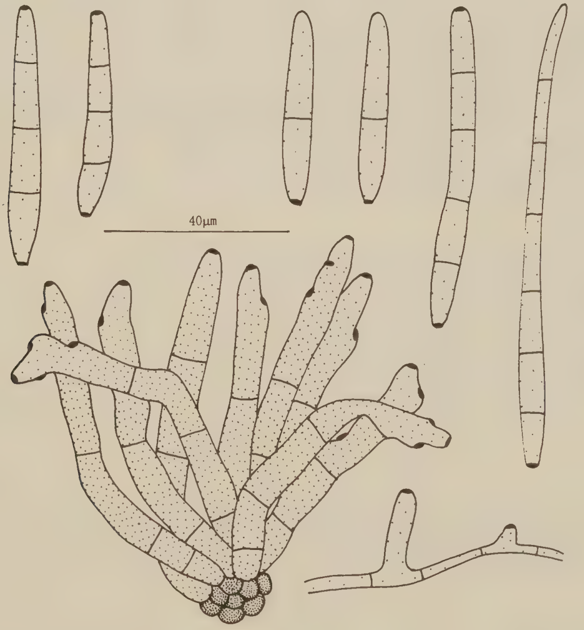

Fig. 2 *Mycovellosiella codonopsis* Y. L. Guo

Leaf spots amphigenous, circular to irregular, 1-5 mm diam., sometimes confluent,

yellowish discolorations or center greyish brown, margin dark grayish brown, with pale yellowish brown halo on the upper surface, yellowish brown, grayish brown to dark grayish brown on the lower surface. Fruiting chiefly hypogenous. Secondary mycelium external: hyphae emerging through stromata, pale olivaceous, branched, septate, 2.2-3.7 µm wide. Stromata none or only a few brown globose cells, substomatal. Conidiophores 2-16 emerging through stomata or arising singly as lateral branches from secondary mycelial hyphae, olivaceous to olivaceous brown, uniform in color, irregular in width, straight to slightly curved, geniculate, not branched or branched, rounded to conical at the apex, 0-4-septate, constricted, 4.3-86.5 × 4.3-8.7 µm. Conidial scars conspicuously thickened, 2.2-3.2 µm wide. Conidia solitary or catenate and in branched chains, obclavate to cylindrical, shorter ones clavate, olivaceous, straight to curved, rounded, obtuse to conical at the apex, obconically truncate at the base, 1-7- septate, sometimes constricted, 22-91 × 4.8-7.6 µm.

*Mycovellosiella isotomae* (Cif.) U. Braun (1993) on *Isotoma longiflora* (L.) Presl. differs from this species in having shorter and narrower conidiophores (15-60 × 2.5-6µm), and subcylindrical, fusiform to obclavate, paler (subhyaline, pale yellowish green to olivaceous), shorter and narrower conidia (15-55 × 2-5µm).

**Pseudocercospora vaccinii** (Kats. & Kob.) Y. L. Guo, comb. nov.
Bas.: *Cercospora vaccinii* Kats. & Kob., Trans. Mycol. Soc. Japan 16(1): 3-6, 1975.
Ref.: Katsuki & Kobayashi (1975: 1-15, Fig. 3).
Type: Taisha, Hikawa, Japan; *Vaccinium bracteatum* Thunb.; T. Kobayashi; Nov. 14, 1973 (not seen).

Fruiting amphigenous. Mycelium immersed and external. Stromata none or small. Conidiophores 6-14 fasciculate or arising singly from the external mycelial hyphae, pale olivaceous brown to pale brown, branched, 1-5-septate, 16-85 × 2.5-5 µm. Conidia cylindrical, cylindric-obclavate to obclavate, pale olivaceous brown, straight to slightly curved, obtuse at the apex, obconically truncate at the base, indistinctly 3-6-septate, 22.5-85 × 3.5-5 µm.

On *Vaccinium* sp.: Yuxi, Yunnan Province (HMAS 79146).

In the Chinese collection on *Vaccinium* sp., the characteristics are very similar to the original description of *Cercospora vaccinii* Kats. & Kob. (conidiophores 7-15 fasciculate, 11-40(-60) × 2.4-4.5 µm, conidia cylindrical-obclavate or narrowly obclavate, pale brown to olivaceous, 38-70 × 2.5-4.5 µm), only the conidiophores are slightly longer.

Sine the conidial scars are indistinct and the conidia are cylindrical, cylindrical-obclavate to obclavate, and coloured, this fungus has to be placed in *Pseudocercospora.*

## ACKNOWLEDGEMENTS

The author especially wishes to express sincere gratitude to Dr. U. Braun and Prof. J. K. Bai for reviewing the manuscript. Thanks are due to Prof. G. S. Zhou for the identification of the host plants and to Ms. X. F. Zhu for inking the drawings.

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## 中国尾孢菌属及其近似属的研究 VI.

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摘要：本文报道 2 个新种：铁线莲菌绒孢 *Mycovellosiella clematidis* Y. L. Guo，生于铁线莲属 *Clematis* sp. 植物上，党参菌绒孢 *Mycovellosiella codonopsis* Y. L. Guo，生于党参属 *Codonopsis* sp. 植物上，1 个新组合：越桔假尾孢 *Pseudocercospora vaccinii* (Kats. & Kob.) Y. L. Guo，生于越桔属 *Vaccinium* sp. 植物上和尾孢菌属的 4 个中国新记录种：鸭跖草生尾孢 *Cercospora commelinicola* Chupp ex U. Braun，秋英尾孢 *Cercospora cosmi* Chiddarwar，凤毛菊尾孢 *Cercospora ratibidae* Ellis & Barthol. 和苦苣菜尾孢 *Cercospora sonchi* Chupp。文中为新种提供了描述并绘图。研究的标本保存在中国科学院微生物研究所菌物标本馆 (HMAS)。

关键词：菌绒孢属，铁线莲菌绒孢，党参菌绒孢，新记录，中国。

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# THE GENUS PODOSPORA IN TAIWAN

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**ABSTRACT**: Twelve species of *Podospora* s. lato have been recorded in Taiwan. The present paper adds seven species new to the country, including *P. formosana* Yei Zeng Wang described as new. Description, comments, illustration of these species, cultural characteristics of some species and a key to total eighteen species collected from Taiwan are provided.

KEY WORDS: *Schizothecium*, *Podospora formosana*, Sordariales, Fungi.

The genus *Podospora* has been studied in detail by several authors (Furuya & Udagawa 1972, Lundqvist 1970, Mirza & Cain 1969). Some new species were added to it (Khan & Krug 1991, Krug & Khan 1989, Lundqvist, 1973, Lundqvist et al. 1999, Udagawa & Ueda 1985). Bell & Mahoney (1995, 1996) recently used "interascal tissue and jacket paraphyses" to replace the traditional use of paraphyses while describing *Podospora* species of the *Schizothecium* group from New Zealand.

Twelve species of *Podospora* s. lato have been recorded in Taiwan (Liou & Chen 1979, Wang 1992, 1994). Among them, *P. absimilis* has been transferred to *Arnium* by Krug & Cain (1972). All specimens cited in this paper were collected from dung samples incubated in moist chambers and examined under fresh condition. Microscopic structures were studied under a light microscope with phase contrast and differential interference contrast lenses, and measurements were made in distilled water mounts. Terms used for description are following Lundqvist (1972). The specimens are deposited at the herbarium of National Museum of Natural Science, Taiwan (TNM).

Key to species of *Podospora* from Taiwan, (inspired by Mirza & Cain 1969).

1. Peridium thin, consisting of swollen cells; hairs agglutinated. .................... 2
   Peridium consisting of angular cells or coriaceous; hairs if present, not agglutinated. 5
2. Asci 8-spored. ........................................................................................ 3

Asci 32 or 64-spored. ........ 4

3. Ascospores more than 30 μm long; upper cauda grooved. ........ *P. curvuloides*

Ascospores 25-29.5 x 17-20 μm; upper cauda not seen. ........ *P. aff. conica*

4. Asci 32-spored; ascospores 20-24 x 10-13 μm. ........ *P. dakotensis*

Asci 64-spored; ascospores 21-24 x 13-15 μm. ........ *P.formosana*

5. Hairs on the necks scattered, short, straight and erected. ........ 6

Hairs not as above. ........ 8

6. Hairs inflated at the apex; ascospores 24-30 x 4-6 μm. ........ *P. inflatula*

Hairs not inflated at the apex. ........ 7

7. Asci 8-spored; ascospores 45-57 x 22-35 μm. ........ *P. fimiseda*

Asci 128-spored; ascospores 17-19 x 10-12 μm. ........ *P. setosa*

8. Hairs tuberculate; upper cauda lyre-shaped. ........ 9

Hairs not tuberculate; upper cauda not lyre-shaped. ........ 11

9. Asci 8-spred. ........ 10

Asci 32-spored. ........ *P. myriaspora*

10. Ascospores 25-34 x 14-16 μm; with a cauda attached at the end of pedicel. ........ *P. argentinensis*

Ascospores 34-39 x 20-22.5 μm; without a cauda attached at the end of pedicel. ........ *P. decipiens*

11. Hairs in tufts. ........ 12

Hairs not in tufts, flexous or lacking. ........ 14

12. Asci 4-spored. ........ *P. anserina*

Asci more than 4-spored. ........ 13

13. Asci 256-spored; ascospores 14-18 x 10-11 μm. ........ *P. curvicolla*

Asci 8-spored; ascospores 24.5 -32 x 15-17.5 μm; pedicel swollen in the middle. ........ *P. prethopodalis*

14. Perithecia glabrous; caudae absent. ........ *P. globosa*

Perithecia not glabrous; ascospores at least with one cauda. ........ 15

15. Ascospores with 4 upper caudae. ........ 16

Ascospores with one upper cauda. ........ 17

16. Ascospores with 4 basal caudae. ........ *P. communis*

Ascospores with one basal cauda. ........ *P. hyalopilosa*

17. Upper and basal cauda composed of numerous filament, and enveloping ascospores. ........ *P. longicaudata*

Cauda not as above; pedicel 44-64 μm long. ........ *P. dolichopodalis*

**Podospora anserina** (Ces. ex Rabenh.) Niessl, Hedwigia 22: 156, 1883.
(recorded by Wang 1992 as *P. pauciseta*)

Specimens examined: Taiwan. Changhua: Yinhangshan, on cow dung, *Y. Z. Wang 95016,* June 7, 1995 (TNM F3439). Hinchu: Linfutcun Zoo, on unknown animal dung, *Y. Z. Wang 94001,* Jan. 5, 1994 (TNM F1659). Pingtung: Kenting, on yellow ox dung, *Y. Z. Wang 92138,* Dec. 12, 1992 (TNM F0582); Nanjenshan, on buffalo dung, *Y. Z. Wang 9269,* Jul. 3, 1992 (TNM F0237); Sheting, on yellow ox dung, *Y. Z. Wang 93057,* Apr. 20, 1993 (TNM F0606); Sheting Park, on buffalo dung, *Y. Z. Wang 9255,* Jun. 26, 1992 (TNM F0203); Yingta Farm, on sheep dung, *Y. Z. Wang 95008,* Feb. 09, 1995 (TNMF3013). Taichung: Tunghai Univ., on goat dung, *Y. Z. Wang 93099,* Sept. 3, 1993 (TNM F1122). Taoyuan: Puhsin, on cow dung, *Y. Z. Wang 93086,* Jul. 21, 1993 (TNM F1000). Fujian. Chinmen: Chinmen, on yellow ox dung, *Y. Z. Wang 94022,* May 12, 1994 (TNMF1978).

Colonies on MEA medium spreading slowly. Mycelium velvety, olivaceous to dark gray, margin smooth, reaching 5.1-5.3 cm in diam. in 14 days at room temperature. Perithecia gradually appearing, semi-immersed to immersed in the agar, numerous. Phialides small, 3-7 x 3-4 µm, with a collarette, phialospores hyaline, subglobose, 1.5-2 µm diam.

Lundqvist (1972) assigned *P. anserina* (Ces.) Niessl as a synonym of *P. pauciseta* (Ces.) Travers. Krug & Khan (1988) still use *P. anserina* for this fungus. However, Lundqvist (pers. comm. 2000) now accepts this name, because *anserina* is a well-known epithet and widely used by cytologists and geneticists.

**Podospora argentinensis** (Speg.) J. H. Mirza & Cain, Can. J. Bot. 47: 2008, 1969.
(Fig. 1)

Perithecia scattered, immersed or semi-immersed, pyriform, 500-600 x 300-400 µm, necks short cylindrical, covered with short black tubercles. Peridium membranaceous, dark brown, polygonal cells 5-10 µm in diam. Asci 8-spored, clavate, 160-200 x 30-40 µm, without apical ring. Paraphyses filiform to ventricose. Ascospores biseriate, ellipsoid, 25-30 x 14-16 µm, pedicel cylindrical 12-17 x 4-6 µm; upper cauda lyre-shaped, 12-18 x 5-8 µm; basal cauda 2-3, attached at the base of pedicel, a single cauda at the end of pedicel, very fugaceous; germ pore eccentric, 1-1.5 µm in diam.

Specimen examined: Taitung: Chihshang, on ox dung, *Y. Z. Wang 94060,* Oct. 19, 1994 (TNM F2575).

This species is close to *P. decipiens,* which has larger ascospores and lacks a cauda at the end of pedicel. The pedicel of this collection is much shorter than those of typical material (35-40 x 6-8 µm, Mirza & Cain 1969). This is a new record for Taiwan.

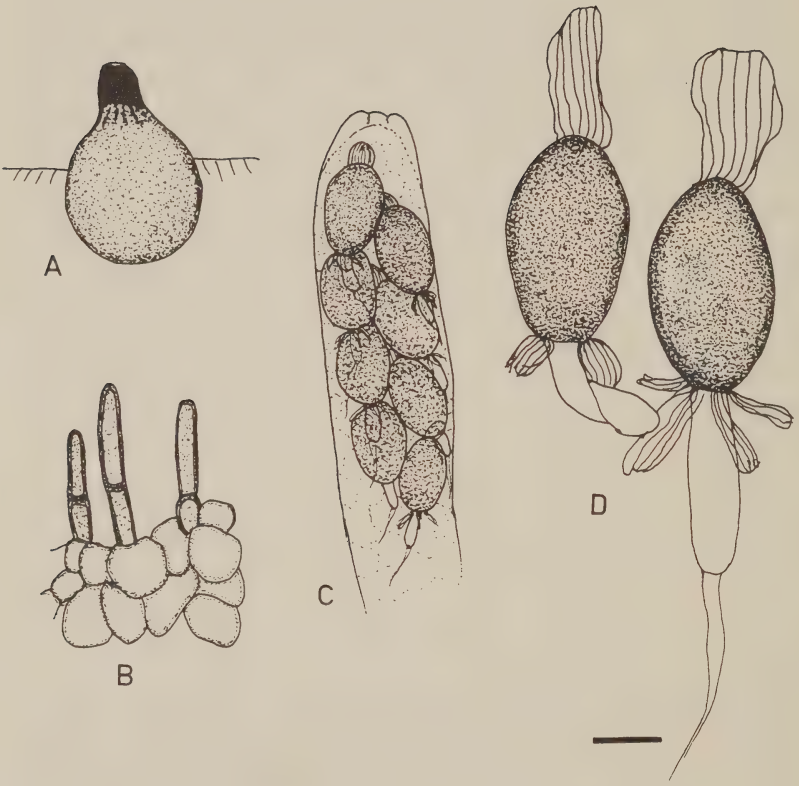

Fig. 1 *Podospora argentinensis*, *Wang 94060* A) Perithecium. B) Tubercular hairs. C) Upper part of an ascus. D) Ascospores. Bar = 200 μm for A, 25 μm for C, and 10 μm for B, D.

**Podospora communis** (Speg. ) Niessl, Hedwigia 22: 156, 1883.
(recorded by Liou & Chen 1979)

Specimens examined: Taiwan. Changhua: Yinhangshan, on cow dung, *Y. Z. Wang 95018,* June 22, 1995 (TNM F3441). Nantou: Chingching Farm, on cow dung, *Y. Z. Wang 92148,* Dec. 22, 1992 (TNM F0583). Pingtung: Kenting, on yellow ox dung, *Y. Z. Wang 92149,* Dec. 22, 1992 (TNM F0584). Taichung: Houli, on horse dung, *Y. Z. Wang 92113,* Oct. 7, 1992 (TNM F0684); Tunghai Univ., on cow dung, *Y. Z. Wang 9233,* May 29, 1992 (TNM F0181); Tunghai Univ., on cow dung, *Y. Z. Wang 95014,* June 7, 1995 (TNM F3437). Taipei: Yangmingshan, Chingtienkang, on ox dung, *Y. Z. Wang 9801,* Jan. 5, 1998 (TNM F7964). Taoyuan: Puhsin, on cow dung, *Y. Z. Wang 93091,* Aug. 1, 1993 (TNM F1009).

Colonies on CMA medium spreading slowly. Mycelium velvety to felty, olivaceous brown, margin irregular, reaching 1.0-1.3 cm in diam. in 14 days at room temperature. Perithecia gradually appearing, immersed to semi-immersed in the agar, numerous. Anamorph not observed.

**Podospora aff. conica** (Fuckel) A. E. Bell. & Mahoney, Mycologia 87: 379, 1995
(Fig. 2)

Perithecia scattered, superficial to immersed, pyriform, 550-750 x 360-550 μm, neck short, black, 110-125 x 90-100 μm. Peridium membranceous, semi-transparent, covered with tufts of agglutinated hairs. Asci 8-spored, clavate, 257-270 x 37-49 μm, apical ring not distinct. Ascospores biseriate, broadly ellipsoid, brown, 25-29.5 x 17-20 μm; pedicel slender, 5-12.5 x 1.5-2.5 μm; upper and basal cauda not observed; germ pore slightly eccentric, 1-2.5 μm in diam.

Specimen examined : Fujian. Chinmen: Chinman, on sheep dung, *Y. Z. Wang 94025,* May 24, 1994 (TNM F2102).

I did not observe the very fugaceous upper and basal caudae which were described by Furuya & Udagawa (1972), and Mirza & Cain (1969). This collection has tufted hairs sized between *P. conica* and *P. squamulosa* (H. & P. Crouan) Niessl. The former has large hair tufts, up to 120 μm long (Lundqvist 1972). It differs from both species by broader spores lacking gelatinous cauda.

**Podospora curvicolla** (G. Winter) Niessl, Hedwigia 22: 156, 1883.
(recorded by Wang 1992)

Specimens examined: Taiwan. Kaohsiung: Fengshan, on goat dung, *Y. Z. Wang 93016,* Feb. 11, 1993 (TNM F0589). Miaoli: Huoyenshan, on rabbit dung, *Y. Z. Wang 93120,* Dec. 17, 1993 (TNM F1443). Pingtung: Kenting, on yellow ox dung, *Y. Z. Wang*

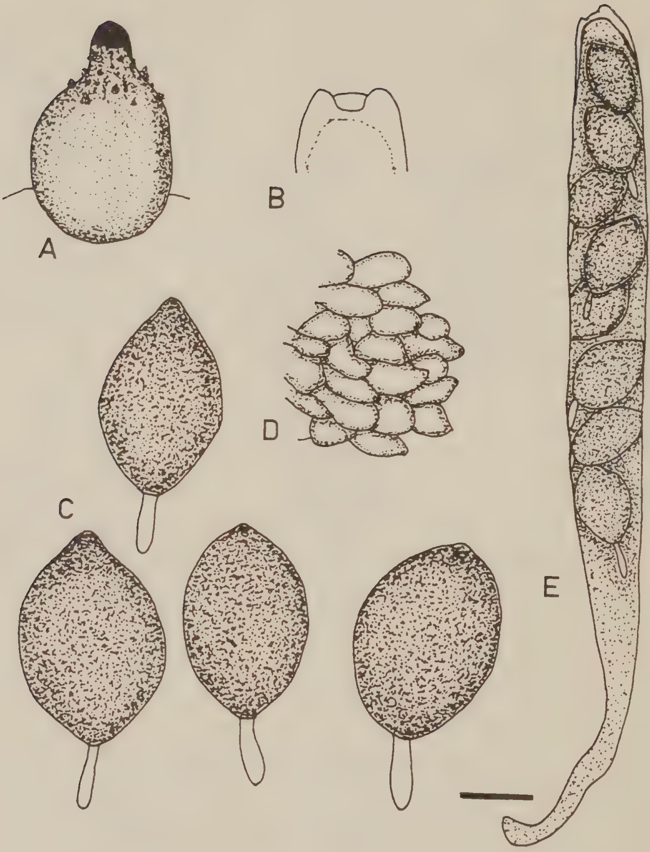

Fig. 2 *Podospora aff. conica*, *Wang 94025* A) Perithecium. B) Tip of an ascus. C) Ascospores. D) Tufted hairs of neck. E) Ascus. Bar = 200 µm for A, 25 µm for E, and 10 µm for B, C, D.

*93063,* Apr. 26, 1993 (TNMF0610); Nanjenshan, on goat dung, *Y. Z. Wang 9268,* July 3, 1992 (TNM F0235). Taichung: Tunghai Univ., on cow dung, *Y. Z. Wang 93042,* Mar. 25, 1993 (TNM F0587). Taipei: Taiwan Univ., on goat dung, *Y. Z. Wang 93089,* July 29, 1993 (TNM F0999). Taitung: Green Island, on goat dung, *Y. Z. Wang 9626,* Apr. 5, 1996 (TNM F4548). Fujian. Chinmen: Chinmen, on sheep dung, *Y. Z. Wang 94051,* Sep. 1, 1994 (TNM F2486).

**Podospora curvuloides** Cain, Can. J. Bot. 40: 453-454, 1962.
(recorded by Wang 1992)

Specimens examined: Taiwan. Nantou: Chingching Farm, on cow dung, *Y. Z. Wang 95012,* Mar. 11, 1995 (TNM F3169). Taipei: Yangmingshan, on cow dung, coll. *Z. C. Chen; Wang 9209,* Mar. 17, 1992 (TNM F0158); Chingtienkang, on ox dung, *Y. Z. Wang 9805,* Jan. 9, 1998 (TNM F7967).

Colonies on MEA medium spreading slowly. Mycelium velvety, olivaceous to dark gray, margin smooth, reaching 0.9-1.0 cm in diam. in 14 days at room temperature. Perithecia gradually appearing, immersed to semi-immersed in the agar, numerous. Anamorph not observed.

**Podospora dakotensis** (Griffiths) J. H. Mirza & Cain, Can. J. Bot. 47: 2016, 1969.
(Fig. 3)

Perithecia scattered or gregarious, superficial, to immersed, narrowly pyriform, 600-700 x 280-300 μm, sparsely covered with flexuous hairs; neck cylindrical, 140-160 x 100-140 μm, black, opaque, covered with tufts of short, agglutinated hairs, composed of thin-walled cells, 3-5 μm in diam. Peridium membranaceous, semi-transparent, olivaceous brown. Paraphyses filiform to ventricose. Asci 32-spored, clavate, 200-240 x 40-48 μm, enlarged when mature, tapering into a long stipe, without apical ring. Ascospores mulitseriate, ellipsoid, olivaceous brown to dark brown, 20-24 x 10-13 μm; pedicel short cylindrical, hyaline, 6-8 x 1.5-2 μm; upper cauda eccentrically attached, 30-40 x 2-3 μm; basal cauda cylindrical, 28 x 1.5-2 μm, extending in water; germ pore apical, 1-1.5 μm wide.

Colonies on CMA medium spreading slowly. Mycelium velvety, pinkish gray, margin smooth, reaching 0.9-1.0 cm in diam. in 14 days at room temperature. Perithecia gradually appearing, superficial to immersed in the agar, numerous. Anamorph not observed

Specimens examined: Taiwan. Nantou: Chingching Farm, on sheep dung, *Y. Z. Wang 93003,* Jan. 05, 1993 (TNM F0703); Chingching Farm, on ox dung, *Y. Z. Wang 9804,* Dec. 05, 1997 (TNM F8284). Taichung: Houli, on horse dung, *Y. Z. Wang 92128,*

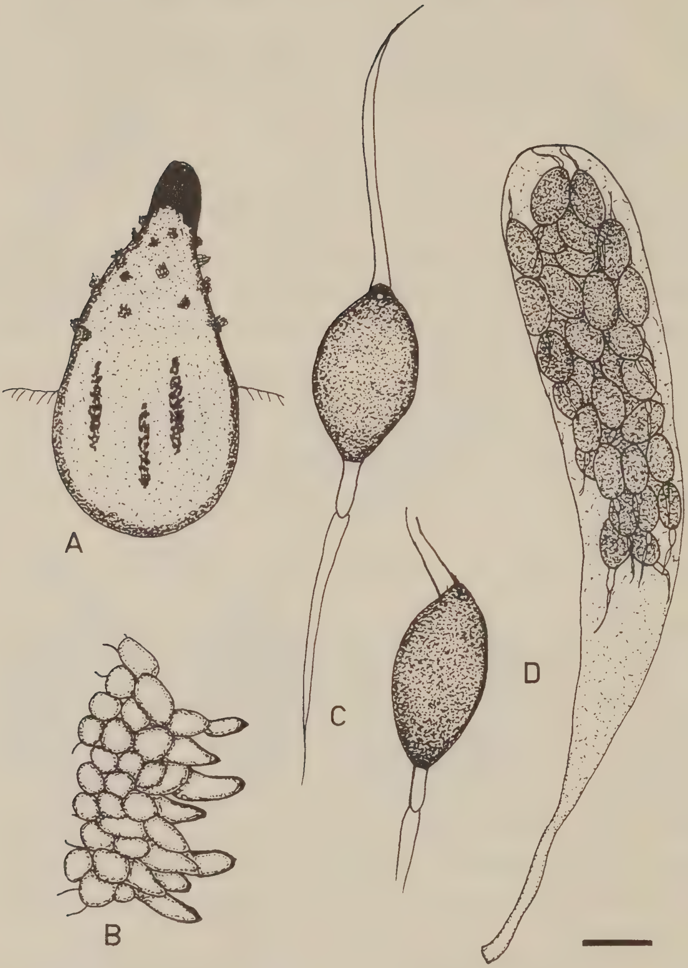

Fig. 3 *Podospora dakotensis*, *Wang 9804* A) Perithecium. B) Tufted hairs of neck. C) Ascospores. D) Ascus. Bar = 200 µm for A, 25 µm for D, and 10 µm for B, C.

Nov. 11, 1992 (TNM F0692).

This species is recognized by the 32-spored asci. It is a new record for Taiwan. Collection *Wang 92128* also has 32-spored asci, but with large perithecia; 1200-1400 x 800-900 μm, and ascospores 24-30 x 15-18 μm. I tentatively assigned this collection under this species.

**Podospora decipiens** (G. Winter ex Fuckel ) Niessl, Hedwigia 22: 156, 1883.
(recorded by Wang 1994)

Specimens examined: Taiwan. Nantou: Chingching Farm, on sheep dung, *Y. Z. Wang 94020,* May 12, 1994 (TNM F1976).

As indicated by Lundqvist (1973), this is a temperate species but occasionally found in tropics at high altitude. This Taiwanese specimen confirms this observation because it was found from dung collected at 1850 m. Krug & Khan (1989) made a same conclusion on East African collections.

**Podospora dolichopodalis** J. H. Mirza & Cain, Can. J. Bot. 47: 2018, 1969.
(Fig. 4)

Perithecia scattered, immersed, pyriform to ovoid, 840-1100 x 700-900 μm, neck black, short cylindrical, 200-250 x 170-250 μm. Peridium membranaceous, olivaceous brown. Asci 8-spored, clavate, with long stipes, 400-425 x 32-70 μm, without apical ring. Paraphyses abundant, filiform to ventricose. Ascospores biseriate, olive-black, ellipsoid, 29-39 x 17-21 μm, pedicel cylindrical, 44-64 x 6-8 μm; upper cauda lash-like, eccentrically attached, 40-61 x 7-10 μm; basal cauda slender, 49-98 x 5-7 μm, extending in water; germ pore apical, 1-2 μm in diam.

Specimen examined: Taiwan. Ilan: Fushan Botanical Garden, on muntjac dung, *Y. Z. Wang 9648,* Nov. 25, 1996 (TNM F5706). Miaoli: Taiwan Provincial Miaoli Agri-industrial Vocational High School, on horse dung, *Y. Z. Wang 9616,* Jan. 30, 1996 (TNM F4473).

This species is characterized by the long pedicels as the species epithet suggests. Description and illustration are based on *Wang 9648*. The pedicels of collection *Wang 9616* are as long as up to 75 μm. Muntjac dung is a new substrate for this species. It is new to Taiwan.

**Podospora fimiseda** (Ces. & De Not.) Niessl, Hedwigia 22: 156, 1883.
(Wang 1994)

Specimens examined: Taiwan. Nantou: Chingching Farm, on cow dung, *Y. Z. Wang 94018,* Apr. 12, 1994 (TNM F1932); *Y. Z. Wang 95001,* Feb. 10, 1995 (TNM F3016);

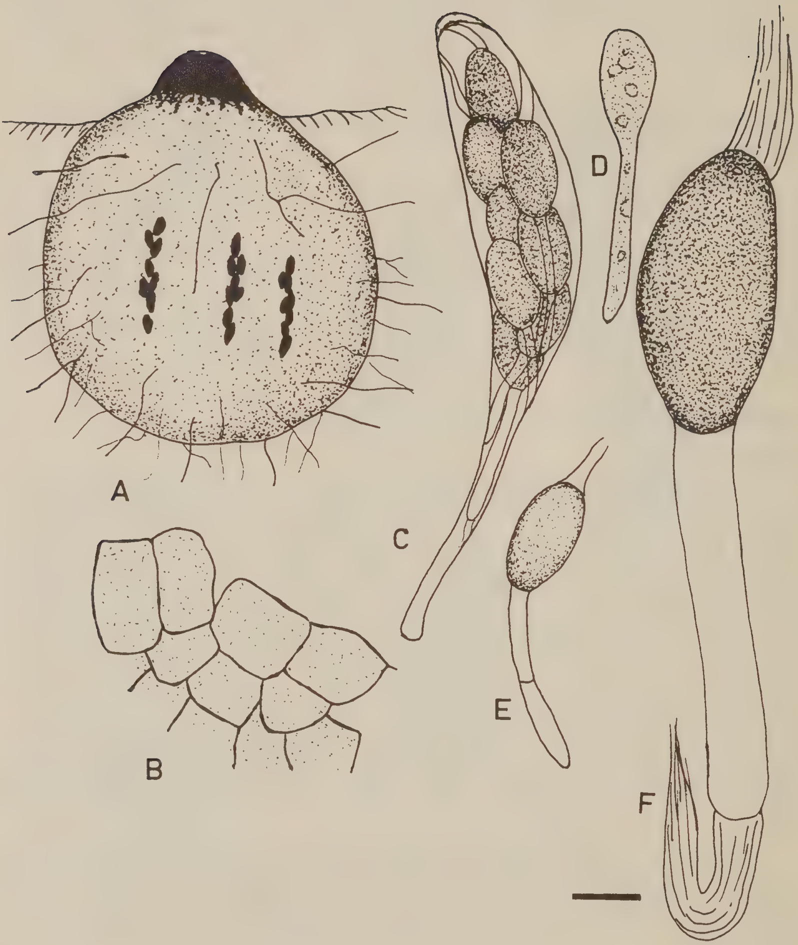

Fig. 4 *Podospora dolichopodalis, Wang 9648* A) Perithecium. B) Cells of peridium. C.) Ascus. D) Young ascospore E) Ascospore. F) Ascospore. Bar = 200 µm for A, 25 µm for C, D, E, and 10 µm for B, F.

on sheep dung, *Y. Z. Wang 9818*, Feb. 13, 1998 (TNM F7978). Pingtung: Tsaopu, on buffalo dung, *Y. Z. Wang 93060*, Apr. 26, 1993 (TNM F0731). Taipei: Yangmingshan; Chingtienkang, on ox dung, *Y. Z. Wang 9727*, Dec. 9, 1997 (TNM F7963).

Colonies on CMA medium spreading slowly. Mycelium white to grayish, septate, mostly submerged, reaching 7.2-8.0 cm diam. in 14 days at room temperature. Perithecia gradually appearing after 21 days, superficial to immersed in the agar, numerous, morphologically similar to those collected from dung. Anamorph not observed.

**Podospora formosana** Yei Zeng Wang sp. nov.
(Fig. 5)

*Perithecia dispersa vel gregaria, superficialia, partim semi immersa ad immersa, anguste pyriformia, 550-820 x 300-460 µm, ostiolata, pilis flexuosis; collum cylindraceum, 100-200 x 80-100 µm, nigrum, opacum, pilis brevibus, agglutinatis, inflatis, 3-5 µm latis. Peridium pseudoparenchymatum, membranaceum, semitranslucidum, olivaceibrunneum. Paraphyses hyalinae, cellulis grandibus. Asci 64-spori, late clavati, 250-280 x 55-115 µm, stipite brevi paulatim attenuato, J-, apice nonannulato. Ascosporae multiseriales, ellipsoideae, primum hyalinae, olivaceibrunneae vel brunneae, (20-)21-24.5 x 13-15 µm, poro germinali apicali 1-1.5 µm lato, pedicello basali brevi hyalino, 5-8 x 1.5-2.5 µm; cauda superior excentrice affixa, 30-49 x 2.5-4 µm, basaliter applanata; cauda basalis cylindraca 30-56 x 1.5-2.5 µm, in aqua prolongans.*

Perithecia scattered or gregarious, superficial, semi-immersed to immersed, narrowly pyriform, 550-820 x 300-460 µm, sparsely covered with flexuous hairs; neck cylindrical, 100-200 x 80-100 µm, black, opaque, covered with tufts of short inflated agglutinated hairs, 3-5 µm wide. Peridium pseudoparenchymatous, membranaceous, semi-transparent, olivaceous brown, with globose to polygonal cells 7.5-25 µm in diam., inner jacket paraphyses with large hyaline, thin-walled cells, 17-25 x 12.5-20 µm. Asci 64-spored, broadly clavate, 250-280 x 55-115 µm, enlarged when mature, tapering into a short stipe, without apical ring. Ascospores multiseriate, ellipsoid, at first hyaline, early septate, olivaceous brown to brown, (20-)21-24.5 x 13-15 µm, with an apical, 1-1.5 µm wide germ pore, and a short hyaline pedicel, 5-8 x 1.5-2.5 µm; upper cauda eccentrically attached, 30-49 x 2.5-4 µm, usually flattened; basal cauda cylindrical, 30-56 x 1.5-2.5 µm, extending in water.

Colonies on CMA medium spreading slowly. Mycelium grayish to olivaceous brown, septate, mostly submerged, reaching 3.5-3.8 cm diam. in 14 days at room temperature. Perithecia gradually appearing, superficial to immersed in the agar,

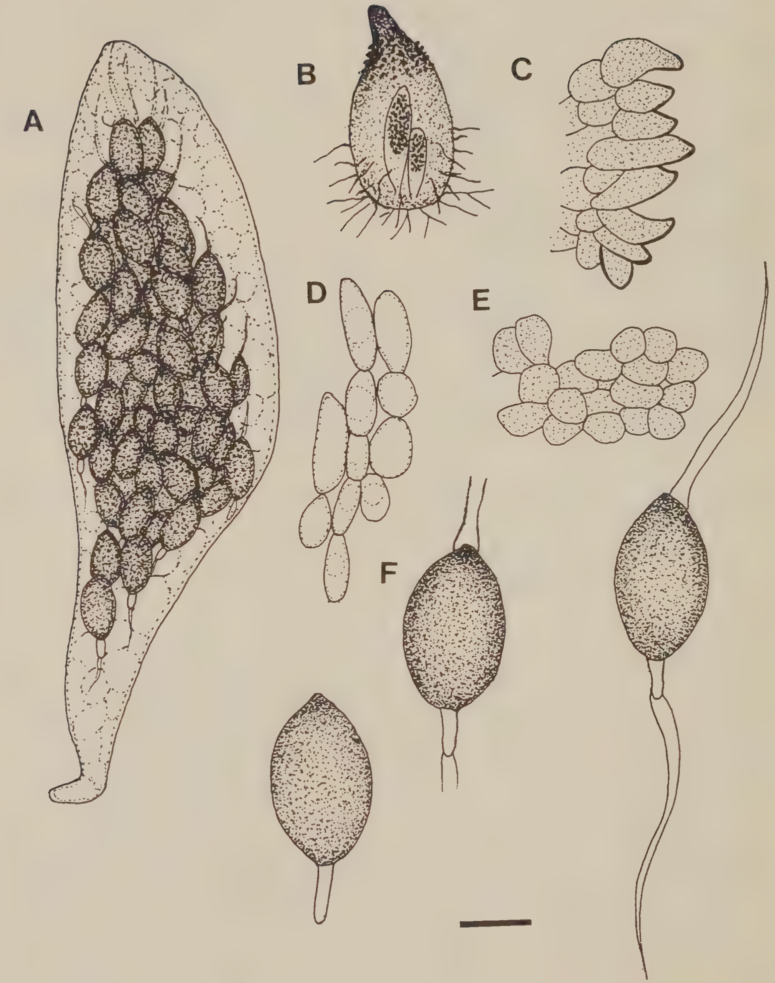

Fig. 5 *Podospora formosana, Holotype* A) Ascus. B) Perithecium. C.) Agglutinated hairs of neck. D) Paraphyses. E) Cells of peridium. F) Ascospores. Bar = 250 µm for B, 25 µm for A, and 10 µm for C, D, E, F.

numerous, morphologically similar to those collected from dung. Anamorph not observed.

*Etymology*: Latinized for the old name of Taiwan.
*Holotype*: Taiwan. Nantou: Chingching Farm, on cow dung, *Y. Z. Wang 9707,* Mar. 10, 1997 (TNM F5715).

*Additional specimens examined:* Taiwan. Nantou: Chingching Farm, on cow dung, *Y. Z. Wang 94012,* Apr. 11, 1994 (TNM F1927); on cow dung, *Y. Z. Wang 9707,* Mar. 10, 1997 (Holotype, TNM F5715); on sheep dung, *Y. Z. Wang 9816,* Feb. 13, 1998 (TNM F7976). Taipei: Yangmingshan; Chingtienkang, on ox dung, *Y. Z. Wang 9806,* Jan. 09, 1998 (TNM F7968).

This species is characterized by short tufted hairs and swollen cells of the perithecia and early septate ascospores, thus making it a member of the *Podospora conica* group ( = *Schizothecium*). Its most prominent feature is the 64-spored asci, which is not known so far in any other species of this group. It is very close to *P. dakotensis*, which differs mainly in having 32-spored asci. Another 32-spored species is *P. alloeochaeta* J. H. Mirza & Cain, which differs in having smaller ascospores. Cultures of *P. formosana* inoculated on sterilized cow dung from other places also grow well and fruit abundantly within 2 weeks.

**Podospora globosa** (Massee & E. S. Salmon) Cain, Can. J. Bot. 40: 406, 1992.
(recorded by Wang 1994)

Specimen examined: Taiwan. Taichung: Tunghai Univ., on cow dung, *Y. Z. Wang 94063,* Oct. 26, 1994 (TNM F2578).

**Podospora hyalopilosa** (R. Stratton) Cain. Can. J. Bot. 40: 460, 1962.
(Fig. 6)

Perithecia scattered, immersed to superficial, pyriform, 600-800 x 360-400 μm, neck black, cylindrical, 120-160 x 100-200 μm, with short, hyaline, septate, hairs. Peridium dark, opaque, with many hyphoid hairs, 1-1.5 μm wide. Asci 8-spored, clavate, apical ring not distinct, 212-230 x 67-87 μm. Paraphyses filiform to ventricose. Ascospores hyaline to olivaceous brown, ellipsoid, 24-34 x 15-18, pedicel cylindrical, 15-30 x 6-7 μm, constricted at the base; upper cauda 3-4, each 3-5 μm wide; basal cauda single, base about 5 μm wide, extending in water; germ pore apical, 1-1.5 μm.

Specimen examined: Pingtung: Kengting, on ox dung, *Y. Z. Wang 9423,* Oct. 7, 1998 (TNMF8943).

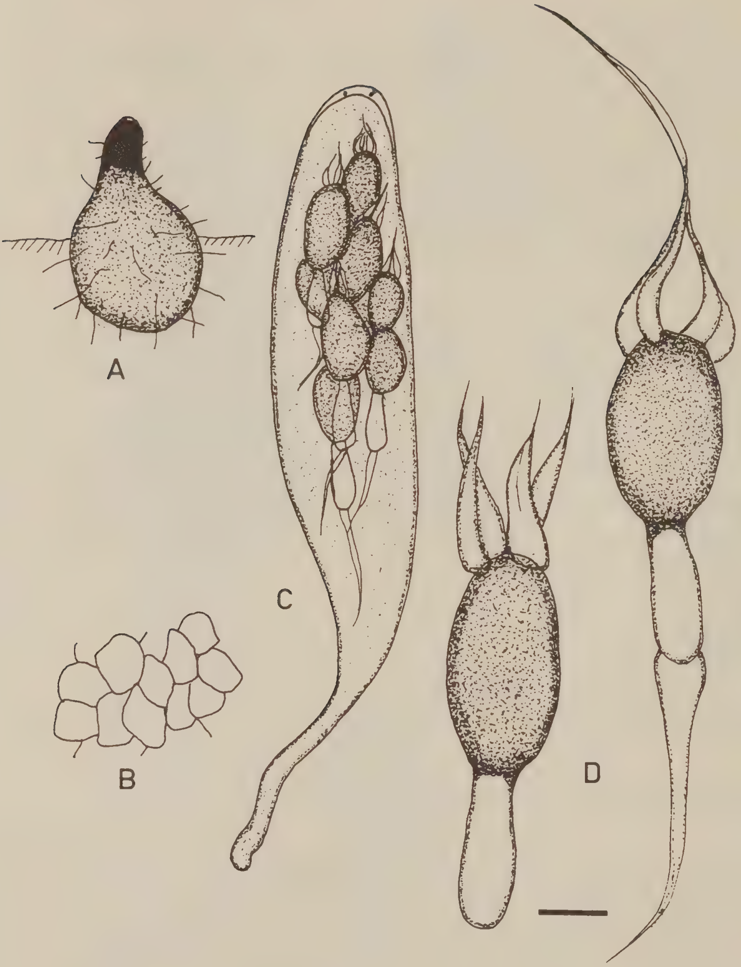

Fig. 6. *Podospora hyalopilosa, Wang 9423* A) Perithecium. B) Cells of peridium. C) Ascus. D) Ascospores. Bar = 200 µm for A, 25 µm for C, and 10 µm for B, D.

The upper caudae on the ascospores are sometimes entangled and elongated together as one single cauda. The species is new to Taiwan.

**Podosproa inflatula** Cain, Can. J. Bot. 40: 454, 1962.
(Fig. 7)

Perithecia scattered, superficial to immersed, pyriform to ovoid, 340-500 x 300-400 μm, neck short cylindrical, black, covered with dark brown hairs. Peridium olivaceous-brown, membranaceous, semitransparent, composed of polygonal cells, 10-20 μm in diam. Hairs light brown, septate, cylindrical, 40-50 x 3-4 μm, with enlarged hyaline tips; 5-7 μm wide. Asci 8-spored, clavate, 185-235 x 28-31 μm, tip with an apical ring 3-4 μm wide. Paraphyses filiform to ventricose, septate, 3-8 μm wide. Ascospores 8-spored, biseriate, olivaceous brown, ellipsoid, 26-32 x 14-15 μm; pedicel cylindrical, hyaline, without content, 24-30 x 4-6 μm; upper cauda eccentrically attached, 30-45 x 4-6 μm, transversely striated; a similar basal cauda at the end of pedicel, 30-40 x 2-4 μm, both extending in water; germ pore apical, 0.7-1 μm in diam.

Colonies on CMA medium spreading slowly. Mycelium pinkish gray, zonate, septate, mostly submerged, reaching 8-9 cm in diam. in 21 days at room temperature. Perithecia gradually appearing, superficial to immersed in the agar, numerous. Immersed perithecia do not have hairs. Anamorph not observed.

Specimen examined: Nantou: Leinwachih, on cow dung, *Y. Z. Wang 2002,* Jan. 16, 2000 (TNM).

This species is characterized by the inflated tips of perithecial hairs, and narrowly ellipsoid ascospores. The upper and basal cauda on the ascospores of this collection are transversely striated, but this characteristic was not described by Cain (1962) and Mirza & Cain (1969). It is a new record for Taiwan.

**Podospora longicaudata** (Griffiths) Cain, Can. J. Bot. 40: 460, 1962.
(recorded by Wang 1992)

Specimens examined: Taiwan. Penghu: Paisha, on ox dung, *Y. Z. Wang 9721,* June 18, 1997 (TNM F7413). Pingtung: Kangtzu, on buffalo dung, *Y. Z. Wang 94040,* Jul. 17, 1994 (TNM F2241); Nanjenshan, on cow dung, *Y. Z. Wang 9264,* June 30, 1992 (TNM F0232). Taitung: Green Island, on goat dung, *Y. Z. Wang 9625,* Apr. 5, 1996 (TNM F4547). Yunlin: Tungshih, on cow dung, *Y. Z. Wang 94075,* Nov. 5, 1994(TNM F2589). Fujian. Chinmen: Chinmen, on cow dung, *Y. Z. Wang 94023,* May 12, 1994 (TNM F1979).

This species is found only within the southern area, and might be a tropical to subtropical species.

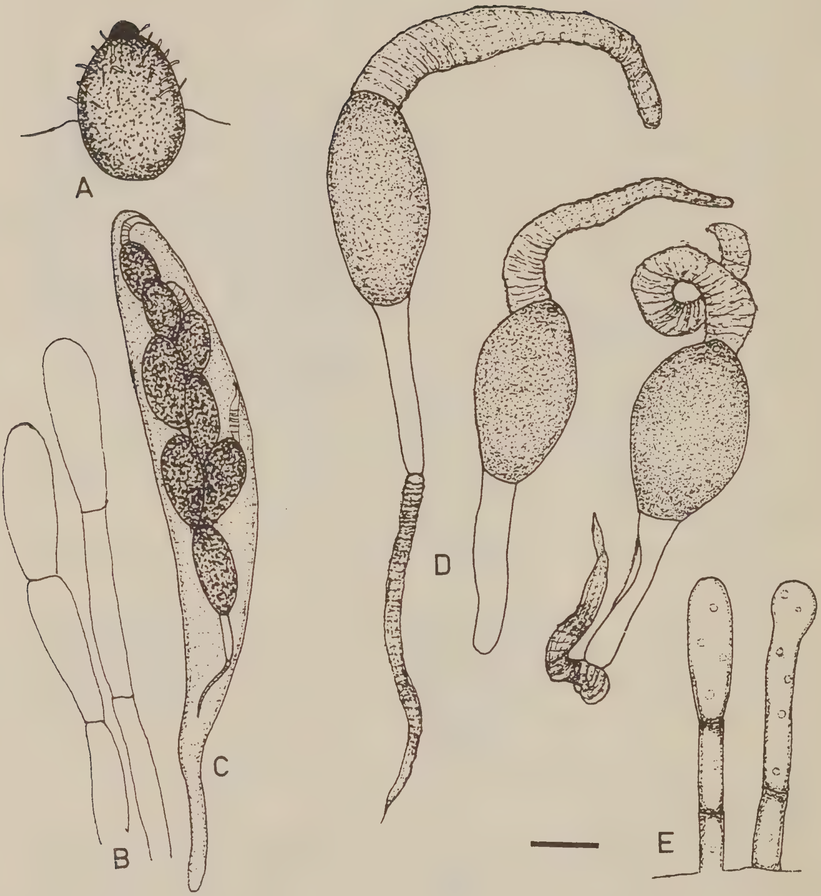

Fig. 7 *Podospora inflatula, Wang 2002* A) Perithecium. B) Paraphyses. C) Ascus. D) Ascospores. E) Perithcial hairs. Bar = 200 μm for A, 25 μm for C, and 10 μm for B, D, E.

**Podospora myriaspora** (H. & P. Crouan) Niessl, Hedwigia 22: 156, 1883.
(recorded by Wang 1994)

Specimens examined: Taiwan. Nantou: Chingching Farm, on cow dung, *Y. Z. Wang 94019,* Apr. 12, 1994 (TNM F1933); on sheep dung, *Y. Z. Wang 9817,* Feb. 13, 1998 (TNM F7977). Taichung: Shenkang. on cow dung, *Y. Z. Wang 92150,* Dec. 23, 1992 (TNM F0752); Tunghai Univ., on cow dung, *Y. Z. Wang 94053,* Sept. 7, 1994 (TNM F2487).

**Podospora prethopodalis** Cain, Can. J. Bot. 40: 458, 1962.
(recorded by Liou & Chen 1979 and Wang 1994)

Specimens examined: Taiwan. Pingtung: Kengting, on ox dung, *Y. Z. Wang 9821,* Sep. 24, 1998 (TNM F9841); on yellow ox dung, *Y. Z. Wang 93062,* Apr. 26, 1993 (TNM F0609); Manchou, on cow dung, *Y. Z. Wang 94054,* Sep. 23, 1994 (TNM F2488); Paoli, on goat dung, *Y. Z. Wang 9643,* Jul. 29, 1996 (TNM F5303). Taitung: Chihshang, on cow dung, *Y. Z. Wang 94041,* Jul. 21, 1994 (TNM F2242).

**Podospora setosa** (G. Winter) Niessl, Hedwigia 22: 156, 1883.
(recorded by Wang 1992)

Specimens examined: Taiwan. Kaohsiung: Chiahsien, on cow dung, *Y. Z. Wang 93121,* Dec. 30, 1993 (TNM F1444); Chiahsien, on cow dung, *Y. Z. Wang 94003,* Jan. 10, 1994 (TNM F1660). Pingtung: Nanjenshan, on goat dung, *Y. Z. Wang 9807,* Jan. 9, 1998 (TNM F7969). Taichung: Chunghsing Univ., on cow dung. *Y. Z. Wang 9252,* June 24, 1992 (TNM F0200); Tunghai University, on cow dung, *Y. Z. Wang 9245,* June 19, 1992 (TNM F0193).

## ACKNOWLEDGEMENTS

The author thanks Dr. N. Lundqvist for advising and critically reviewing the manuscript; Mr. W. N. Chou for Latin description, and people who assisted in collecting samples. This study is partly supported by NSC 89-2311-B-178-002.

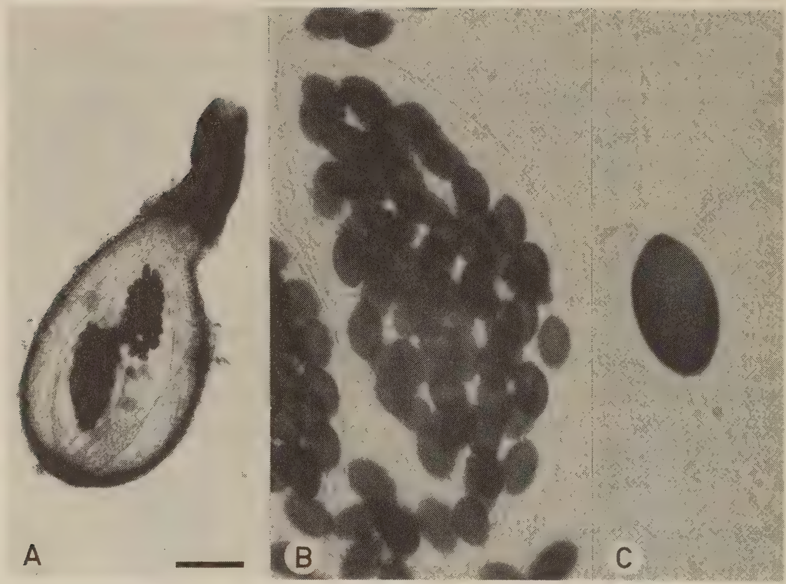

Fig. 8 *Podospora formosana, Holotype* A) Perithecium. B) Ascus. C) Ascospore. Bar = 250 µm for A, 25 µm for B, and 10 µm for C.

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# THE GENUS LECANOSTICTA FROM NUEVO LEÓN, MEXICO

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## ABSTRACT

An account of the species of the genus *Lecanosticta* found on pines in Nuevo León is presented. *Lecanosticta longispora*, a fungus collected on *Pinus culminicola*, is described as a new species. A Nomenclator and a key for identification of species of *Lecanosticta* occurring in Nuevo León are also provided.

**Key words**: *Lecanosticta*, *Pinus*, *Leptomelanconium*, *Gloeocoryneum*, Forest Pathology

## INTRODUCTION

*Lecanosticta* is an important genus for forest pathologists, since *Lecanosticta acicola*, the anamorph of *Mycosphaerella dearnesii* Barr, causes a widespread and severe needle blight on several species of pine. Gibson (1978) mentioned this species as one of the most important foliage diseases of pine in North America. The EPPO (1996) classifies this species as an A2 quarantine pest, and because of its adaptability in North America, suggests that it presents a considerable risk for Europe. Evans (1984) studied this genus on Central American pines and included some Mexican pines. The same author described a new species of *Lecanosticta*, (*L. gloeospora*) from Nuevo León on *Pinus pseudostrobus* Lindley.

The State of Nuevo León, Northeast Mexico, has a great diversity of pine species. Eleven species of *Pinus* occur in the State. Since little research has been made on the fungi associated with these species, the present study gives the results from a survey of the genus *Lecanosticta* on pines in Nuevo León. A new species is described from *Pinus culminicola* Andresen & Beaman.

## MATERIAL AND METHODS

Collections were made of pine needles bearing *Lecanosticta*-like fructifications. Notes of the host, damage and other ecological features were taken. Specimens were brought to laboratory where fungi were examined using the usual mycological techniques.

All the specimens examined have been deposited in the Herbarium of the Facultad de Ciencias Forestales, UANL (CFNL). Some specimens of *Lecanosticta* species from IMI and the Institut fuer Pflanzenschutz im Forst, Braunschweig, Germany, were also examined.

## RESULTS

**Lecanosticta acicola** (Thüm.) Sydow

This species was found on *Pinus ayacahuite* Ehrenberg ex Schlechtendal, *P. cembroides* Zuccarini, and *P. halepensis* Miller. The most severe damage was observed on the last species, which is planted at the College's experimental forest for research purposes. The specimens examined showed similar dimensions to those obtained from the typespecimen of *L. acicola*.

Specimens examined

USA: IMI 91340 type of *Cryptosporium acicolum* Thümen Flora 1878 p. 78. [*Lecanosticta acicola* (Thüm.) Syd.], South Carolina, Aiken, col. H.W. Ravenel. In *Pinus variabilis* Lamb. 1876. in Herbario Univ. Padova; IMI 104312 *Lecanosticta acicola*, Durham, North Carolina, 20.04.1940, *Pinus palustris*, F.A. Wolf. GERMANY: M253 *Lecanosticta acicola* Ortsbereich Murnau, B.D.R., 10.1994, *Pinus mugo*, L.Pehl; M257 *Lecanosticta acicola* Ortsbereich Murnau, B.D.R., 10.1994, *Pinus mugo*, L.Pehl. HONDURAS: IMI 281582 *Lecanosticta acicola* Montaña de Celaque, Lempira, 18.10.1981, *Pinus ayacahuite*, H.C. Evans. MEXICO: IMI 281599 *Lecanosticta acicola*, Acaxochitlan-Zacatlán, Puebla, 9.05.1983, *Pinus patula*, H.C. Evans; CFNL 318 *Lecanosticta acicola* El Orito, Galeana, Nuevo León, 30.07.1987, *Pinus cembroides*, 2300 m.s.l., J.G. Marmolejo; CFNL 1204 *Lecanosticta acicola* Cerro del Potosí, Galeana, Nuevo León, 29.08.1992, *Pinus ayacahuite*, 3000 m.s.l., J.G. Marmolejo; CFNL 329 *Lecanosticta acicola* Pablillo, Galeana, Nuevo León, 25.08.1989, *Pinus cembroides*, 2000 m.s.l., J.G. Marmolejo; CFNL 1203 *Lecanosticta acicola* Cerro del Potosí, Galeana, Nuevo León, 20.06.1992, *Pinus ayacahuite*, 3000 m.s.l., J.G. Marmolejo; CFNL 319 *Lecanosticta acicola* El Orito, Galeana, Nuevo León, 7.06.1990, *Pinus cembroides*, 2400 m.s.l., J.G. Marmolejo; CFNL *Lecanosticta acicola* Bosque Escuela, Iturbide, Nuevo León, 19.01.1995, *Pinus halepensis*, 1600 m.s.l., J.G. Marmolejo; CFNL 1276 *Lecanosticta acicola* Bosque Escuela, Iturbide, Nuevo León, 20.08.1994, *Pinus halepensis*, 1500 m.s.l., J.G. Marmolejo

**Lecanosticta gloeospora** H.C. Evans

This species, described by Evans from Nuevo León, was found only on *Pinus pseudostrobus associated with* a light needle discoloration. It differs from *Lecanosticta acicola* by its smaller and dark brown conidia which have a thick mucilaginous sheath.

Specimens examined

MEXICO: IMI 283812 *Lecanosticta gloeospora* Iturbide-Galeana, Nuevo León, 16.05.1983, *Pinus pseudostrobus*, H.C. Evans (Type); CFNL 505 *Lecanosticta gloeospora*, La Palapa, Bosque Escuela, Iturbide, Nuevo León, 27.09.1990, *Pinus pseudostrobus*, 1500 m.s.l., J.G. Marmolejo.

***Leptomelanconium pinicola*** (Berk. & M.A. Curtis) R.S. Hunt
≡ *Stilbospora pinicola* Berk. & M.A. Curtis
=*Gloeocoryneum cinereum* (Dearn.) Weindlm.

This species was collected on *Pinus arizonica* Engelmann var. *stormiae* Martinez and on *P. hartwegii* Lindley, associated with a needle blight. Evans (1984) placed this species under *Lecanosticta* based on the basis of conidiogenesis and conidial septation. He proposed the name *Lecanosticta cinerea*, but this combination is not valid, because no basionym was given. Hunt (1984) considered *Gloeocoryneum cinereum* a synonym of *Stilbospora pinicola* Berk. & M.A. Curtis, stated that there are no differences between this genus and *Leptomelanconium* and proposed the name *Leptomelanconium pinicola*. According to Peterson (1981), this species can cause a severe blight resembling *Dothistroma* blight. This species was already known from Mexico on *Pinus cooperi* Blanco from Durango. (Hunt, *op. cit.*).

Specimens examined

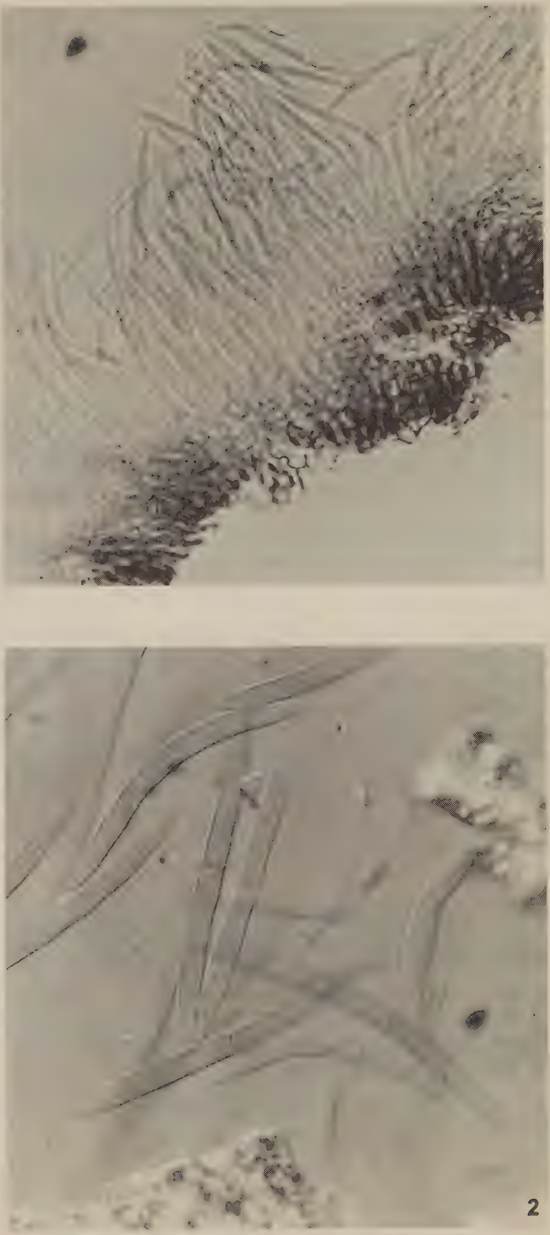
MEXICO: CFNL 503 Aserradero Ejido 18 de Marzo, Galeana, Nuevo León, 15.11.1986, *Pinus arizonica*, 2100 m.s.l., J.G. Marmolejo; CFNL 507 Santa Clara, Galeana, Nuevo León, 13.03.1991, *Pinus arizonica*, 1800 m.s.l., J.G. Marmolejo; CFNL 1212 Cerro del Potosí, Galeana, Nuevo León, 20.06.1992, *Pinus hartwegii*, 3000 m.s.l., J.G. Marmolejo

***Lecanosticta longispora*** Marmolejo sp. nov.
Conidiomata acervulis *Lecanostictae acicolae* similiter, differo conidiis longioribus, 37-52 x 2,5-3,5 µm

Conidiomata acervularia, hypophylla, olivaceo-brunnea, subepidermalia, erumpentia, elliptica, 150-200 um lata, 80-110 µm alta, peridermio laciniato rumpentes. Stroma basale 50-70 µm profundum, cellulis pseudoparenchymaticis, parietibus crassiusculis, brunneis, polyedricis, 4-9 µm diam. compositum. Conidiophora hyalina usque ad pallido-brunneola, ramosa, septata. Conidia brunnea usque ad pallido-spadicea, cylindrica, maximam partem recta, interdum leviter curvata, apice rottundato, basi truncata, parietibus duplicatis, verrucata minuta, 1-3 septata, 37-52 x 2,5-3,5 µm, in cirro olivaceo mucilagino superficie acervuli liberata.
Hab. In foliis brunneis *Pini culminicolae* Andresen & Beaman, Mexico, Nuevo León, Galeana, Cerro del Potosí, legit J.G. Marmolejo, 6. VI. 1993, in herb. CFNL depositus (Holotypus)

Acervuli hypophyllus, olive brown, subepidermal, becoming erumpent, elliptical, 150-200 µm wide, 80-110 µm tall, opening through a slit. Basal stroma 50-70 µm deep, composed of pseudoparenchyma of thick walled, brown, polyhedric cells 4-9 µm wide. Conidiophore hyaline to pale brown, branched, septate. Conidia brown to light brown, cylindrical, mostly straight, sometimes slightly curved, with a rounded apex and a truncate base, double-walled, minutely verrucose, 1-3-septate, 37-52 x 2.5-3.5 µm, released on the surface of the acervulus in an olive-green mucilaginous cirrus.

Specimen examined: Mexico, Nuevo León, Galeana, Cerro del Potosí, 3200 m.s.l., Marmolejo 6.06.93 on *Pinus culminicola* (Holotype: CFNL)



Figs 1-2: *Lecanosticta longispora*. 1. Conidiomata. 2. Conidia

## KEY TO THE SPECIES OF *LECANOSTICTA* KNOWN FROM NUEVO LEON

1.- Conida usually no more than 35 µm long......2
1.- Conidia more than 35 µm long ..............*Lecanosticta longispora*
2.- Conidia dark brown, 10.5-14.5 long ...... *Lecanosticta gloeospora*
2.- Conidia light brown, 15-35 µm long ...... *Lecanosticta acicola*

## NOMENCLATOR FOR THE KNOWN SPECIES OF *LECANOSTICTA*

*Lecanosticta* Syd. in Syd. & Petr. Annales Mycologici 20: 211, 1922

*Lecanosticta pini* Syd. in Syd. & Petr. Annales Mycologici 20: 211, 1922 (= *Lecanosticta acicola*)

*Lecanosticta acicola* (Thüm.) Syd. in Syd. & Petr. Annales Mycologici 22: 400, 1924

*Lecanosticta gaubae* (Petr.) Arx & Constant. in Arx Proceedings, Koninklijke Nederlandse Akademie van Wetenschappen. Series C 86(1): 34, 1983

*Lecanosticta cinerea* (Dearn.) H.C. Evans Mycological Papers 153: 88, 1984 (not validly published)

*Lecanosticta gloeospora* H.C. Evans Mycological Papers 153: 92, 1984

## ACKNOWLEDGMENTS

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Hunt, R.S., 1985. *Leptomelanconium pinicola* comb. nov. and associated needle blight of pines. Can. J. Bot. 63: 1157-1159
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# TYPE STUDY OF LEPISTA RICKENII (TRICHOLOMATALES) AND DESCRIPTION OF L. PANAEOLUS VAR. PAXILLOIDES VAR. NOV.

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**Summary**. After the revision of the holotype of *Lepista rickenii* Singer, we conclude that this is most probably contaxic with *L. multiforme* (Romell) Gulden. The new taxon *L. panaeolus var. paxilloides* is proposed and described, found in a mixed mediterranean *Quercus ilex-Pinus pinea* forest in Spain. This last probably represents, at least in part, some authors' concept of *L. rickenii*. Drawings of microcharacters for both taxa are given.

**Key words**: *Lepista rickenii, L. multiforme, L. ovispora, L. panaeolus* var. *paxilloides*, Tricholomatales, taxonomy, *Quercus ilex, Pinus pinea*, Spain.

## INTRODUCTION

To compare *Lepista rickenii* Singer with our new taxon proposed, we requested on loan the holotype of this species from LE After the study of this type we concluded that Singer's taxon is probably conspecific with the circumboreal *L. multiforme* (Romell) Gulden. The original description by SINGER (1948), complemented with the study of the holotype, which is kept in good state, show that both taxa share similar morphological features.

We repeat the original description of *L. rickenii*, supplemented with data from our morphological studies of the holotype, with additional taxonomic comments. *Lepista panaeolus var. paxilloides* Esteve-Rav. & Villarreal is proposed as a new taxon, which corresponds, at least in part, to the concept of some modern descriptions of *L. rickenii* (e.g. BON, 1983, 1997; BALLERO & CONTU, 1988, 1991) or *L. panaeolus* (Fr.) P. Karst. (e.g. ZUCCHERELLI, 1993). The new taxon is probably widespread in mediterranean areas, where it can be found in grassy vegetation of *Quercus* or *Pinus* woods.

## MATERIAL AND METHODS

The type collection of *L. panaeolus* var. *paxilloides* has been deposited in (AH), University of Alcalá Herbarium, Spain. The microscopical study of the samples was made using $NH_4OH$ (2 %), congo red (2 %) and Melzer's reagent. Line drawings of the figures were made with the help of a camera lucida. Microphotographs were obtained with a Zeiss-950 SEM. Basidiospore measurements were obtained following the method proposed by HEINEMANN & RAMMELOO (1985). Authors' abbreviations follow KIRK & ANSELL (1992).

## ***Lepista rickenii*** Singer, Sydowia 2: 26-27 (1948)
## (= *Tricholoma panaeolum* s. Ricken, *fide* Singer, *loc. cit.*)
## (Figs. 1, 2, 5 A-D)

### Original diagnosis:

*Pileo tabacino ("Havana 131" Séguy), dein pallidiore, saepe concentrice guttulato, interdum subzonato marmoreoque atque sordide alutaceo-pallido siccando, convexo et subumbonato, dein depresso et saepe subumbonato in depressione infundibuliforme; superficie haud viscosa, glabra, laevi; sed ad marginem primum involutum albopubescente, usque ad 244 mm. lato - Lamellis pallide brunneolo-albidis, confertis, 10-10.5 mm. latis, sinuato-adnexis vel decurrentibus, subcrenulatis; sporis in massa pallidissime sordide roseolis (pallidioribus quam sporae Rhodopaxilli sordidi); sporis sub microscopio hyalinis, sublaevibus (in solutione Melzeri sub lente immersa minutissime asperulatis), ellipsoideis, uno latere (interiore) applanatis, 6-7.5 x 3.8-4 μ; basidiis 25-33 x 5.8-7.5 μ, tetrasporis; hyphis tramatis hymenophoralis paulum intertextis (tramate regulari vel subregulari), fibuligeris. - Stipite albo et subsericeo ad apicem, gradatim magis concolori pileo basin versus et fibrilloso-striatulo, plerumque basin versus incrassato, flexuosa vel curvato, semel in centuriebus ramoso, cavo, usque ad 110 mm. longo, usque ad 26 mm. lato. - Carne sordide alba, hygrophana in cortice; hyphis omnibus fibuligeris. - Caespitose subconnateque (30-40 individua), in orbem crescentes, in pratis, autumno. Typus in Instituto Cryptogamico Academiae Scientiarum U.S.S.R., Leningrad conservatus est (R.Singer, prope Rajajoki, U.S.S.R., Septembri 26, 1940, no. 5-823). - Observatio: A. Ricken Lepistam panaeolum cum L. Rickenii comixit in diagnose, sed elementum conspicuius descriptionis est L. Rickenii.*

Additional macro and microscopic data obtained by the revision of the holotype are the following:
Basidiomata (5-6) growing fasciculate from a common stipe base. Pilei plano-convex to slightly depressed at the center, brown-tobacco coloured,

surface smooth, sometimes with small darker spots at the center. Stipe longer than pileus diameter, concolorous, with aeriferous white fibrils at the apex. Gills subdecurrent, beige-yellowish. Smell of dry grass (hay), present (after more than 50 years since it was collected !).

Spores (Fig. 5 C) L x w = 5.5-6.49-7.2(-8.5) x 3.5-3.97-4.5(-5) µm, Q = 1.55-1.63-1.79(-1.84)-n = 21, ellipsoid to subcylindrical, frequently in tetrads, cyanophilous, very slightly roughened under the optical microscope, with very low warts (verruculose) under the S.E.M. (Fig. 2). Basidia (Fig. 5 B) 25-35 x 6.5-7.5 µm, clavate, 4-spored, clamped, without siderophilous granulation. Hymenial cystidia absent. Pileipellis (Fig. 5 A) of repent, mainly parallel hyphae 2-5 µm wide, embedded in a thin pellicle of gelified material, with scarce terminal cylindrical to subclavate cells, with yellow-brown parietal pigment ; subpellis with cylindrical elements, 5-12 µm wide, with encrusting pigment in thin transverse lines. Hymenophoral trama regular to subregular, made up of elements 2-7 µm wide. Stipitipellis (Fig. 5 D) constituted of regularly septate cylindrical hyphae, 4-7 µm wide, with parietal and encrusting pigment, with numerous narrow, cylindrical, hyaline terminal cells, 2-4 µm wide. Clamp-connections present at all septa.

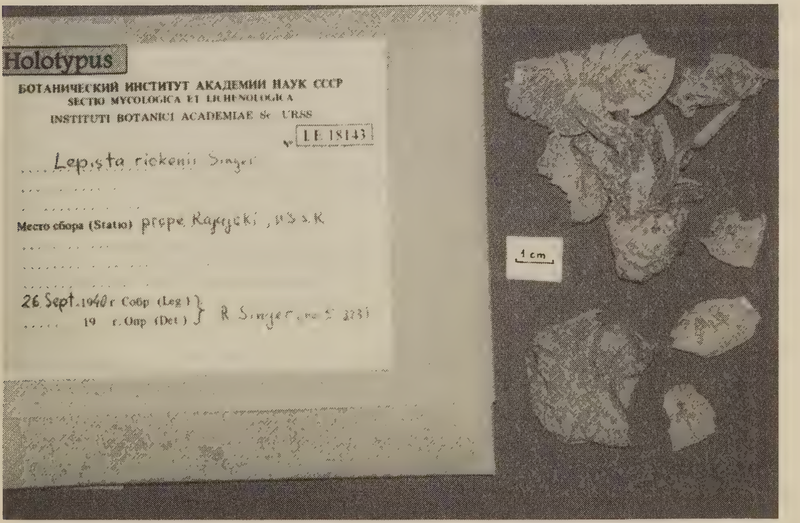
Holotypus
БОТАНИЧЕСКИЙ ИНСТИТУТ АКАДЕМИИ НАУК СССР
SECTIO MYCOLOGICA ET LICHENOLOGICA
INSTITUTI BOTANICI ACADEMIAE Sc URSS
LE 18143
Lepista rickenii Singer
Место сбора (Statio) prope
26 Sept. 1940 г. Сбор (Leg.)
19 г. Опр. (Det.)
R. Singer
1 cm

**Fig. 1.** *Lepista rickenii* Singer (*Holotypus*, LE 18143).

**Discussion**. According to the revision of the holotype, both macro and microscopic features correspond rather well with GULDEN's (1983) concept of *L. multiforme* (Romell) Gulden, and to a lesser extent with that of *L. ovispora* (J.E. Lange) Gulden. The fasciculate habit, never greyish but uniformly tobacco-brown pilei, smell and verruculose, nearly smooth spores, fit reasonably well within the range of variation of both taxa.

In the original diagnosis, SINGER (*loc. cit.*) already mentioned the fasciculate basidiomata, and the boreo-alpine habitat, which seem to be characteristic features of *L. multiforme*. GULDEN (*loc. cit.*) has concluded that both *L. polycephala* Harmaja and *Clitocybe polygonarum* Laursen, O.K. Mill. & H.E. Bigelow are conspecific with *L. multiforme*, after having studied the type collection of the first and typical material of the latter.

Some slight discrepancies, however, between Singer's description and Gulden's are both the smell and the wrinkled warts of the spores described by the latter and not mentioned by the first. Singer does not mention the coumarinic smell which seems to be typical in old specimens of *L. multiforme* (with longeval basidiomata); however, in the type collection, a typical smell of hay is kept, after more than 50 years since it was deposited in (LE). The possibility that Singer based his original description on the observations of both *L. multiforme* and *L. ovispora* should not be excluded and might explain why these discrepancies exist between his description and the type-material. The spore ornamentation shown under the S.E.M. by GULDEN (1983, Fig. 5) for *L. multiforme*, with apically collapsed warts, could be possibly related with the degree of pigmentation during the ripening process, according to the studies carried out by PEGLER & YOUNG (1974); on the other side, both British authors have also observed this phenomenom in other *Lepista* species.

The taxonomic status of *L. ovispora* (J.E. Lange) Gulden is somewhat problematic. This taxon has been traditionally interpreted as a fasciculate *Lyophyllum* species with smooth spores, but no siderophilous basidia were found in the material studied under this name by GULDEN (*loc. cit.*; 1992) from England and Norway, who has also considered *L. fasciculata* Harmaja conspecific. In fact, *L. multiforme* and *L. ovispora* are very close taxa, and the differences pointed out by Gulden are based exclusively on the smell and stipe colour; these taxa have been also recognized as different species by CHRISTENSEN (1997), but Gulden (pers. comm.) is not very certain if they should be treated at different species level. Microscopically, the spores of *L. ovispora* in comparison with those of *L. multiforme*, as shown in GULDEN (*loc. cit.*), seem to be more ornamented, but the spore size in both is overlapping. Moreover, a close taxon fitting within those *Lyophyllum* taxonomical concepts, i. e. presence of siderophilous basidia and smooth spores, is also present in our Continent, as remarked by BON (1997: 107).

Selected photographs of *L. multiforme* can be consulted in GULDEN & al. (1985: 19), LAURSEN & al. (1976: figs. 5-8) - as *C. polygonarum*-, MOSER & JÜLICH (1992: Lepista 3) and CHRISTENSEN (*loc. cit.*: 20).

A comparison between the descriptions of *Tricholoma panaeolus* by RICKEN (1915: n° 1041, taf. 95, fig. 1), and that of SINGER (*loc. cit.*) of *L. rickenii* (= *T. panaeolus* s. Ricken, *fide* Singer) show clearly that both taxa are different, and there are at least three clear discrepancies referred to: I) the guttulate pileus, II) smell and III) habit; the taxon described by Ricken has a raphanoid smell, is not fasciculate, shows a pileus spotted at the margin and sinuate-subdecurrent lamellae; these characters are typical for *L. panaeolus s. l.* Some modern European authors have usually followed Singer's interpretation, and considered *L. rickenii* very close to *L. panaeolus* (Fr.) P. Karst. (e.g. BON, 1983, 1997; NOORDELOOS & KUYPER, 1995). However, this last species differs in several features, mainly in habit, colours, guttulate pileus and the more coarsely ornamented spores, which show prominent, rounded and wide warts (PEGLER & YOUNG, *loc. cit.* ).

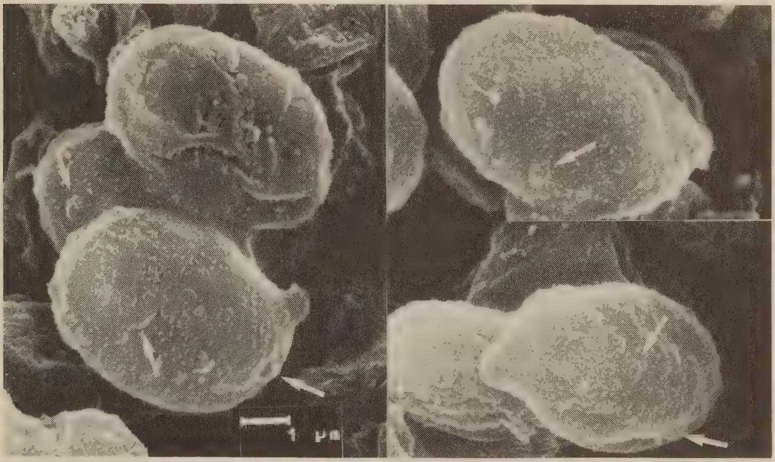

**Fig. 2.** *Lepista rickenii* Singer (*Holotypus*, LE 18143). Spores under S.E.M. showing sparse small warts (arrowheads).

## ***Lepista panaeolus* var. *paxilloides* Esteve-Rav. & M. Villarreal *var. nov.***

(Figs. 3, 4, 5 E-H)

*A varietate typica differt lamellis decurrentibus et contextus fuscus. Holotypus in AH 22120.*

Basidiomata (Fig. 3) gregarious, normally in circles, either isolated or fasciculate. Pileus 15-60 mm, convex to applanate, sometimes obtusely umbonate, with persistently strongly involute margin, dry or moist in humid weather, minutely felty to subsquamulose at the centre in old specimens, not hygrophanous, not translucently striate, dark grey (Mu 10R 5-6/1) to brownish-grey (Mu 10R 4-5/2-4) at margin, darkening slightly to dusky-red (Mu 10R 3/4) in old washed specimens, often with darker brown drop-like spots at the margin. Lamellae very crowded, very narrow, thin, deeply arquate-decurrent, never sinuate, greyish to purple-greyish (Mu 10R 5-6/2-4), with entire, concolorous edge. Stipe 20-70 x 4-15 mm, cylindrical to slightly clavate, sometimes slightly broadened at the apex, longitudinally striate with aeriferous dark greyish fibrils, pubescent-fibrillose at the apex, pale gray 10R 6/1, with white basal mycelial tomentum. Context firm, at first white, becoming fuscous or even dark greyish with age. Smell farinaceous with an acidulous component. Taste not recorded. Spore print light cream (in AH 25299).

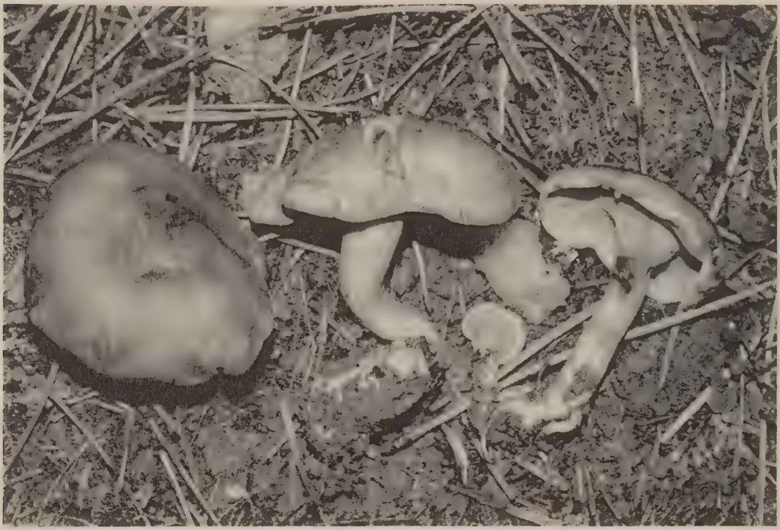

**Fig. 3.** *Lepista panaeolus* var. *paxilloides* Esteve-Rav. & Villarreal (*Holotypus*, AH 22120). Habit showing strongly arcuate-decurrent lamellae.

Spores (Fig. 5 G) L x w = 5.50-6.11-7.19(-7.50) x 3.50-3.94-4.50) µm, Q = 1.42-1.55-1.69(-1.71)-n = 21, ellipsoid to subcylindrical, frequently in tetrads, cyanophilous, roughened under the optical microscope, with prominent warts (verrucose) under the S.E.M. (Fig. 4). Basidia (Fig. 5 F) 28-36 x 6.5-9 µm, clavate, 4-spored, clamped. Hymenial cystidia absent. Pileipellis (Fig. 5 E) of repent, mainly parallel hyphae 5-12 µm wide, not gelified, with scarce terminal cylindrical to subclavate cells showing irregular terminal ends, containing yellow-brown parietal and also vacuolar pigment; subpellis with cylindrical elements, -160 x 5-12 µm, with parietal and vacuolar pigment, plus encrusting in thin transverse lines. Hymenophoral trama regular to subregular, made up of elements 4-10 µm wide. Stipitipellis (Fig. 5 H) constituted by regularly septate cylindrical hyphae, 4-8 µm wide, with parietal and encrusting pigment, with numerous cylindrical, flexuose, sligthly thick-walled, hyaline terminal cells, 4-8 µm wide. Clamp-connections present at all septa.

Habitat: terrestrial, in grassy thermophilous mediterranean forests with *Quercus ilex* subsp. *ballota* (Desf.) Samp. (= *Q. rotundifolia* Lam.) and/or *Pinus*.

Material studied. SPAIN. TOLEDO, Pinar de Almorox, 19-XI-1996, *F. Esteve-Raventós, C. Sánchez & M. Villarreal*, (AH 22120 ***Holotypus***). BURGOS, locality unknown, 27-X-1999, in grassy vegetation near *Quercus ilex* forest, *F.D. Calonge & F. Esteve-Raventós*, AH 25299.

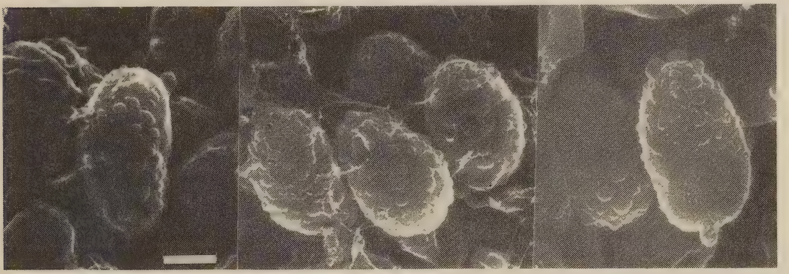

**Fig. 4**. *Lepista panaeolus* var. *paxilloides* Esteve-Rav. & Villarreal (*Holotypus*, AH 22120). Spores under the S.E.M. showing prominent obtuse warts and smooth suprahilar plage.

**Discussion**. *Lepista panaeolus* (Fr.) P. Karst. (= *L. luscina s. auct. pl.*) is a relatively well known and widely distributed species in the European continent, where it is usually described and photographed in mycological books or guides. However, it has been interpreted in different ways by some authors (see KÜHNER's, 1976 comments on this subject), or considered a taxon with a large variability in its macrocharacters, which has led to the recognition of many infraspecific taxa (see BOHUS, 1970, 1979).

*Paxillus lepista* Fr. is accepted by most authors (e.g. SINGER, 1943, 1986; HORAK, 1968; KÜHNER, *loc. cit.*; BON, 1983, 1997; NOORDELOOS & KUYPER, 1995) as the type species of the genus *Lepista* (Fr.) W.G. Sm.; today, it is generally admitted that *P. lepista* is conspecific with *L. densifolia* (J. Favre) Singer & Clémençon, a species with typical decurrent lamellae. Special emphasis is given nowadays to lamellar attachement in the infrageneric delimitation of this genus, as has been considered by BON (*loc.cit.*).

*L. panaeolus* is a member of the subgenus *Rhodopaxillus* (Maire) Bon, owing to its "tricholomoid" habit and adnate, emarginate to subdecurrent lamellae. In young fruitbodies, lamellar insertion is always

*Tricholoma*-like, and in old, large specimens the lamellae may become subdecurrent, but always sinuate-arquate near the stipe, forming a depressed circle around it. We doubt that our new taxon fits into the limits of subgenus *Lepista* Fr. in spite of the distinctly decurrent, pointed lamellae. All other characters come closer to *L. panaeolus s. l.* than to any other species of this last section. BON (1997: 102) comments on that the taxonomic position of *L. rickenii* Singer can be questioned, and that his own interpretation of this species could constitute a transitional taxon between both subgenera.

*L. panaeolus* var. *paxilloides* already shows in young fruitbodies arquate-decurrent, never emarginate lamellae, a character which is also present in *L. rickenii s.* Bon. Only the spores, which BON (*loc. cit.*) describes with an ondulose outline (*Rhodocybe*-like), are a very contrasting character between both, because in var. *paxilloides* they are clearly verrucose, as PEGLER & YOUNG (*loc. cit.*) define for *L. panaeolus* group. BALLERO & CONTU (*loc. cit.*) have followed Bon's concept of *L. rickenii*.

Our new taxon is macroscopically characterized by: I) the narrow, thin and strongly arquate-decurrent pointed lamellae with greyish-pinkish reflection, II) a permanently convex pileus with involute margin, III) dark greyish-brown guttulate pileus, IV) concolorous stipe (in some specimens longer than pileus diameter) V) farinaceous smell with a fruity component and VI) darkening flesh; microscopically, both taxa are very similar.

NOORDELOOS & KUYPER (*loc. cit.*) differentiate *L. panaeolus* and *L. rickenii* by the pileus color, darker in the second, but sharing both taxa the same type of pigment and lamellar attachment. However, we think that a certain variation of the color in *L. panaeolus* is exhibited in nature, this character mainly influenced by environmental factors (especially after heavy rainfall) or the degree of maturation of the fruitbodies (normally darker in young fruitbodies), or the true existence of genuine darker variants; the size of the fruitbodies within one species is also variable, and the long lasting fruitbody development of many *Lepista* species may favour the appearance of large pilei in the basidiomata. We agree with both dutch authors that some vacuolar pigment is also present in the pileipellis of *L. panaeolus*, as could be tested in typical material of this species from (AH) studied by us.

The photographic plate of *L. luscina* in ZUCCHERELLI (1993) illustrates a form of our taxon rather well, with the typical dark colours of the fruitbodies and the strongly arquate decurrent, pointed and narrow lamellae.

A related species is *L. tomentosa* M. Moser, which is characterized by the trichodermial pileipellis and black spotted stipe at the apex (MOSER & JÜLICH, 1992: Lepista 2-below), and found recently in Spain (BALLARÀ, 1993).

In North America, according to the revision of BIGELOW (1982), the closest taxa seem to be *Clitocybe brunneocephala* H.E. Bigelow (= *Tricholoma harperi* Murrill) which has sinuate, adnate to subdecurrent

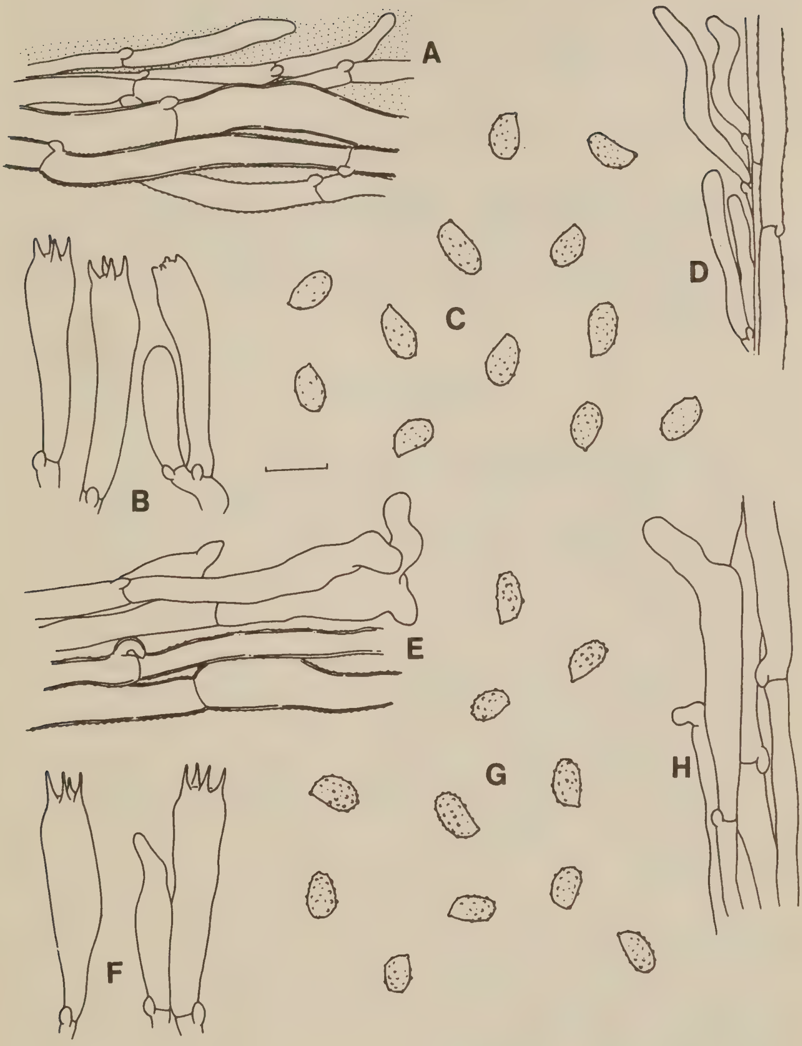

**Fig. 5**. *Lepista rickenii* Singer (*Holotypus*). A, pileipellis; B, basidia; C, spores; D, stipitipellis. *Lepista panaeolus* var. *paxilloides* Esteve-Rav. & Villarreal (*Holotypus*). E, pileipellis; F, basidia; G, spores; H, stipitipellis. Bar = 10 µm.

lamellae, a pleasant smell and different habit, whereas *C. panaeoliformis* (Murrill) H.E. Bigelow & A.H. Sm. and its var. *westii* (Murrill) H.E. Bigelow & A.H. Sm. have an anise smell and sinuate lamellae respectively. On the other hand, it seems strange that a widespread and well known taxon as *L. panaeolus(= L. luscina)* has not been recorded in this continent up to now.

## ACKNOWLEDGEMENTS

We are indebted to Dr. A. Kovalenko for the loan of the holotypus of *L. rickenii* from (LE). Also to Dr. G. Gulden (Botanical Garden and Museum, Oslo, Norway) for her kind suggestions and to Dr. M. Moser (Institut für Mikrobiologie, Innsbruck, Austria) , for critical revision and improving of the manuscript.

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MYCOTAXON

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# ACANTHOGYMNOMYCES, A NEW GENUS OF GYMNOTHECIAL ASCOMYCETES WITH SETAE AND SULCATE ASCOSPORES

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## ABSTRACT

*Acanthogymnomyces terrestris* gen. et sp. nov., a gymnothecial ascomycete from Japanese soil, is described and illustrated. It is characterized by brownish ascomata with numerous long straight setae, cylindrical to pyriform asci and hyaline to pale-colored, oblate ascospores with an equatorial rim and a sulcate ornamentation. The fungus is tentatively accommodated in the Eoterfeziaceae. The new combination *A. princeps* (*Spiromastix princeps* Udagawa et Uchiyama) is proposed.

Key words: ascomycetes, soil fungi, systematics.

During a continuing survey of onygenalean ascomycetes collected in soil samples from various localities of Japan and overseas countries, an interesting gymnoascaceous isolate was described as *Spiromastix princeps* Udagawa et Uchiyama (Udagawa and Uchiyama, 1999), a new species of the genus *Spiromastix* (Kuehn and Orr, 1962). The fungus is quite distinct from other species of the genus in having two kinds of ascomatal appendages and oblate ascospores with a broad equatorial rim and finely sulcate ornamentation.

After the publication, ascomatal clusters of an apparently undescribed ascomycete which resembles *S. princeps* except for lighter color and numerous numbers of long appendages were macroscopically observed growing on an isolation agar plate of the soil sample collected from Kasama City in Japan. Each ascoma was found to contain cylindrical or clavate asci on a clump of basal cells and surrounding paraphysis-like sinuous hyphae as the centrum. The seta-like appendages of the ascomata are reminiscent of those produced by some of *Lasiobolidium* in the Eoterfeziaceae (Malloch and Cain, 1971; Malloch and Benny, 1973; Locquin-Linard, 1983; Moustafa and Ezz-Eldin, 1989; Yaguchi et al., 1996) There are several other characters in common with *Lasiobolidium*, such as cylindrical to clavate, very thin-walled, evanescent asci and small, hyaline, oblate ascospores without a germ pore. On the contrary *Lasiobolidium* differs from this fungus in producing cleistothecial ascomata which have a distinct peridium either composed of coalescing hyphae or with a narrow layer of pseudoparenchymatous cells. Among these characters, the ascus morphology specially links this fungus to members of the Eoterfeziaceae rather than the Onygenaceae.

A comparison with *S. princeps* indicated that the two fungi are congeneric. Ascospores of this fungus are likewise characterized by being hyaline to pale yellowish brown, oblate, with a rather thick equatorial rim and a finely sulcate ornamentation. However, *S. princeps* differs in the shape and size of ascomatal setae (described as elongate appendages in *S. princeps*), the shape and size of the asci and the size of the ascospores. On account of these new findings, it would seem appropriate to place these fungi in a separate genus rather than in *Spiromastix*, in the Onygenaceae. Thus this fungus is described as a new gymnothecial genus and species, and a new combination of *S. princeps* to the new genus is proposed.

## TAXONOMY

*Acanthogymnomyces* Udagawa et Uchiyama, gen. nov.

Ascomata superficialia, dispersa vel confluentia, sine stromate, plus minusve globosa, non ostiolata, brunnea vel atrobrunnea, setosa, sine peridio; centrum flavum, ex cellulis basalibus, ascis aggregatis et hyphis circumdatis composito. Setae ascomatum brunneae, rectae vel parum undulatae, ex centro radiatim exorientes, non ramosae, incrassatae, leves, aseptatae, ad apicem acutae. Asci unitunicati, octospori, cylindracei, clavati vel pyriformes, interdum ovoidei, brevi-stipitati, evanescentes, non apparato apicali praediti. Ascosporae unicellulares, oblatae, hyalinae vel pallescentes, crista aequatoria praeditae, tenuiter ornamentatae.

Coloniae restrictae, floccosae, flavae vel armeniaceae vel olivaceae. Mycelio vegetativo ex hyphis halinis ramosis septatis tenuibus composito.

Species typica: *Acanthogymnomyces terrestris* Udagawa et Uchiyama.

Etymology: Greek, *akanthodes* = thorny, and *gymnomykes* = naked

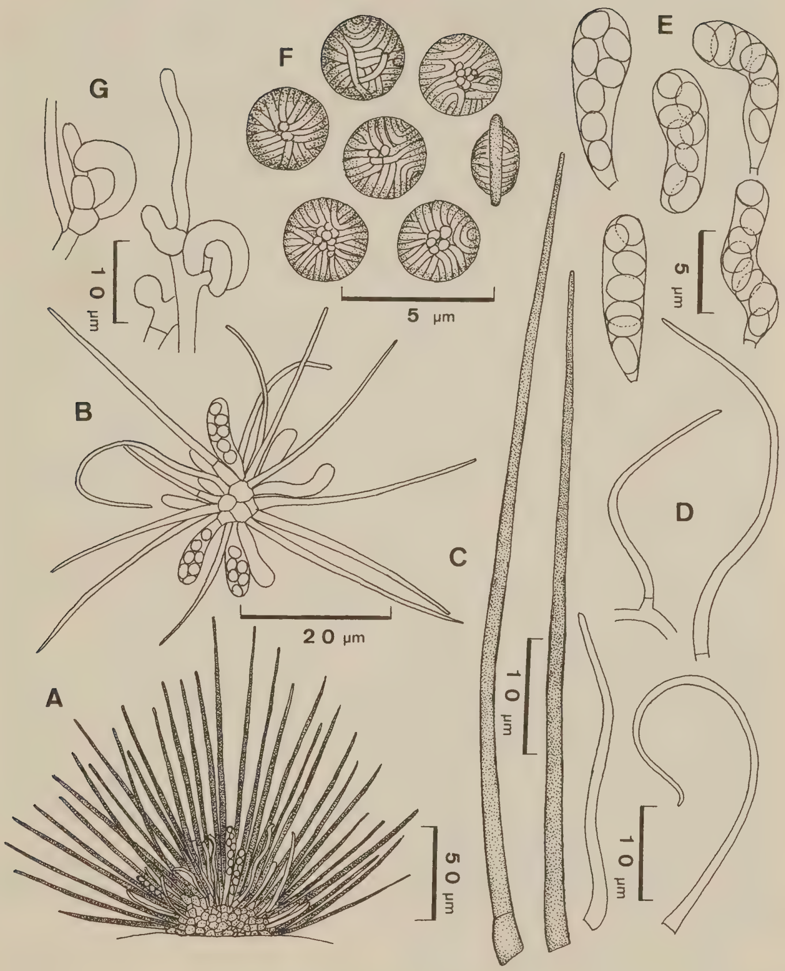


Fig. 1. *Acanthogymnomyces terrestris* (SUM 3162).
A. Ascoma. B. Initial stage of ascoma showing a young centrum. C. Setae. D. Surrounding hyphae of asci. E. Asci. F. Ascospores. G. Ascomatal initials.

fungus, referring to the characteristics of the ascomata with numerous setae.

Ascomata superficial, scattered or confluent, non-stromatic, more or less globose, non-ostiolate, brown to dark brown, covered with long setae, without distinct peridium; centrum yellow, composed of somewhat irregularly elongate basal cells, a cluster of asci and surrounding sinuous hyphae. Ascomatal setae brown, straight or slightly undulate, radially arising from the centrum, unbranched, thick and smooth-walled, aseptate, with a pointed tip. Asci unitunicate, 8-spored, cylindrical, clavate, pyriform, or sometimes ovoid, short-stipitate, thin, evanescent, without apical apparatus. Ascospores one-celled, oblate, hyaline to pale-colored, with a thick equatorial rim, showing fine ornamentations.

Colonies restricted, floccose, pale yellow to light yellow or olivaceous. Vegetative mycelium consisting of hyaline, branched, septate, thin-walled hyphae.

Type species: *Acanthogymnomyces terrestris* Udagawa et Uchiyama.

*Acanthogymnomyces terrestris* Udagawa et Uchiyama, sp. nov. Figs. 1-9

Ascomata superficialia, dispersa vel saepe confluentia, non ostiolata, globosa vel subglobosa, 150-200 µm diam cum setis peripheralibus, dilute brunnea vel brunnea, sine peridio; centrum dilute flavum, ex cellulis basalibus et ascis aggregatis et hyphis circumdatis composita; hyphae circumdatae hyalinae vel dilute brunneae, sinuatae vel incurvatae vel contortae, 20-50 × 1-1.5 µm, simplices vel uniramosae, leves, pro parte maxima aseptatae, versus apicem acuminatae. Setae ascomatum numerosae, ex centro radiatim orientes, brunneae, rectae, 50-120 × 1.5-2 µm, incrassatae et leves, aseptatae, superne gradatim angustatae et pallescentes, ad apicem acutae. Asci octospori, cylindracei vel clavati, saepe curvati vel contorti, 7-12 × 2.5-4.5 µm, brevi-stipitati, tenues, evanescentes. Ascosporae uniseriatae vel irregulariter uniseriatae, hyalinae vel dilute flavo-brunneae, oblatae, 2.5-2.8 × 1.4-1.6 µm, crista aequatoria praeditae, anguste sulcatae et ad polos tuberculatae.

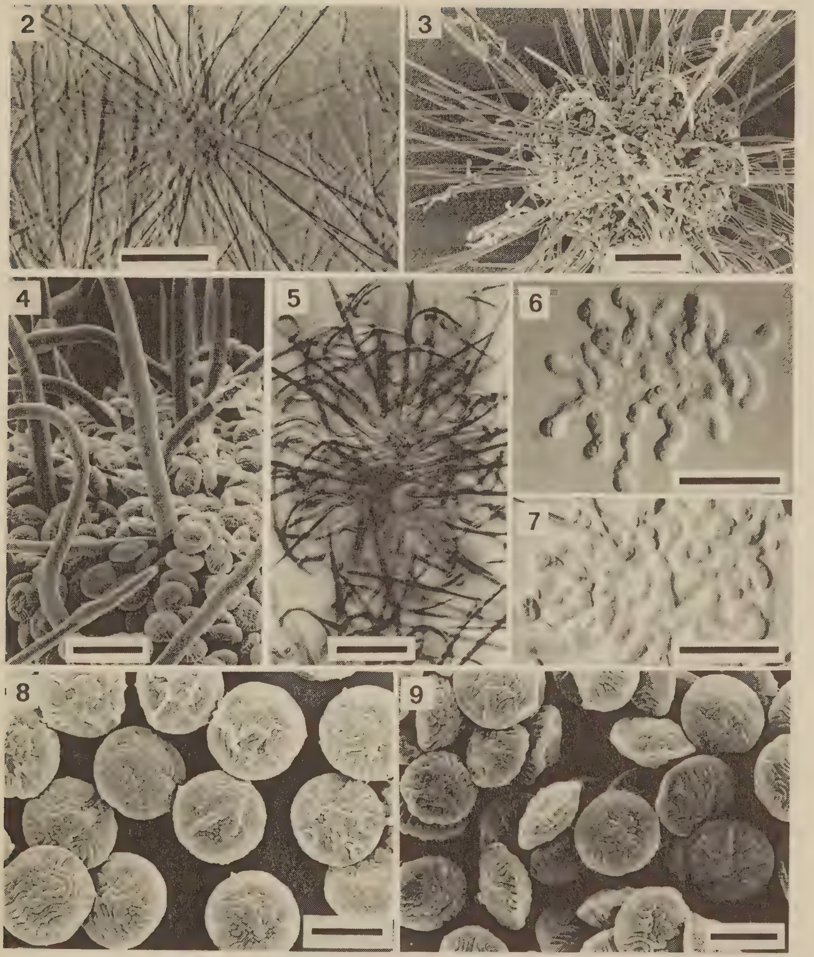
Mycelio vegetativo ex hyphis hyalinis ramosis levibus septatis 1-3 µm diam composito. Anamorphosis abest.

Coloniae in "PCA" restrictae, floccosae, fere planae, ex mycelio coacto tenuiter constantes, dilute flavae vel stramineae, abundantibus ascomatibus formantes; reversum dilute flavum vel sulphureum.

Holotypus: SUM 3162, colonia exsiccata in cultura ex solo sylvae, Ibaraki, in Japonia, 8. VII. 1998, a S. Uchiyama isolata et ea collectione fungorum Musei et Instituti Historiae Naturalis, Chiba (CBM).

Etymology: Latin, *terrestris* = on the ground, referring to the habitat.

Ascomata superficial, scattered or often confluent, non-ostiolate, small, globose to subglobose, 150-200 µm in diam incl. setae, light brown to brown,



Figs. 2-9. *Acanthogymnomyces terrestris* (SUM 3162).
2. Young ascoma showing a centrum. 3. Ascoma with numerous setae (SEM). 4. Surface of ascoma showing mass of ascospores without a peridium (SEM). 5. Ascomatal centrum showing setae and surrounding hyphae. 6, 7. Asci in fascicled arrangement. 8, 9. Ascospores (SEM). Scale bars: 2, 3, 5, 6, 7 = 20 μm; 4 = 5 μm; 8, 9 = 2 μm.

covered with long setae, fragile in age, maturing within 21 d, without distinct peridium, with a pale yellow centrum consisting of a cluster of asci and surrounding hyphae which are borne from a clump of irregularly elongate basal cells, hyaline to pale brown, sinuous to incurved or contorted, 20-50 × 1-1.5 μm, gradually tapering toward a pointed tip, unbranched or once branched, smooth-walled and aseptate except the base. Ascomatal setae numerous, about 35-100 in number per the ascoma, radially arising from the centrum, brown, straight, 50-120 × 1.5-2 μm, unbranched, thick and smooth-walled, aseptate, gradually tapering and paling toward a pointed tip. Asci borne directly from the cells of the centrum tissue, 8-spored, cylindrical or clavate, often curved or contorted, 7-12 × 2.5-4.5 μm, short-stipitate, thin, evanescent, without an apical apparatus. Ascospores uniseriate or irregularly uniseriate, hyaline to pale yellowish brown, oblate, 2.5-2.8 × 1.4-1.6 μm, with a rather thick equatorial rim, finely sulcate with linear ridges over the almost entire surface, often with tuberculate projections in the poles, smooth by optical microscopy.

Vegetative mycelium consisting of hyaline, branched, smooth-walled, septate, 1-3 μm diam hyphae; ascomatal initials consisting of a few short hyphal side branches, coiled or contorted, becoming surrounded by hyphae arising from neighboring cells. Anamorph lacking.

Colonies on PCA growing restrictedly, attaining a diam of 13-14 mm in 21 d at 25°C, floccose, almost plane, consisting of a thin mycelial felt, Pale Yellow (M. 2A3, after Kornerup and Wanscher, 1978) or Straw (Rayner, 1970); ascomata abundantly produced into loose aerial hyphae; margins entire, rather narrow; exudate scattered, clear; reverse and agar Pale Yellow to Light Yellow (M. 4A3-2A4) or Pale Luteous to Sulphur Yellow (R). Colonies on OA growing somewhat slower than on PCA but retaining the same general pattern, compactly felty, Light Yellow to Light Orange (M. 4A4-5A5) in color due to the formation of abundant ascomata borne on the felt; reverse Orange to Deep Orange (M. 5A7-6A7), with surrounding agar highly colored in Light Yellow (M. 3A5) or Pale Yellow shades.

At 37°C, growth is nil.

Holotype: SUM 3162, a dried culture isolated from forest soil, Sashiroyama, Kasama-shi, Ibaraki Pref., Japan, 8 July 1998, col. S. Uchiyama (CBM).

The taxonomic placement of this fungus is controversial because its cylindrical ascus characteristics do not completely fit those of any of the recognized genera in the Onygenales or the Eurotiales (Benny and Kimbrough, 1980; Currah, 1985, 1994; Arx, 1987). Although the ascomata are gymnothecial and without distinct development of hyphal or membranaceous peridium, other characters led us to place it in the

Eoterfeziaceae G. F. Atkinson. Malloch and Cain (1971) described the Eoterfeziaceae as characterized by hyaline to reddish-brown ascocarps, globose to cylindrical asci, hyaline, thin-walled, oblate to ellipsoidal ascospores. Among the Eoterfeziaceae, the ascomata in all species of *Lasiobolidium* are long appendaged indicating a relationship with this fungus in which the ascomata are more setose. In all species of both genera, the structure and fascicled arrangement of the asci are also similar.

Further studies in molecular systematics are necessary to draw final conclusions about its relationships to the other gymnothecial or cleistothecial ascomycetes.

## NEW COMBINATION

*Acanthogymnomyces princeps* (Udagawa et Uchiyama) Udagawa et Uchiyama , comb. nov.

≡*Spiromastix princeps* Udagawa et Uchiyama, Mycoscience 40: 291-305. 1999. (Basionym).

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# PLEUROTUS ERYNGII VAR. ELAEOSELINI VAR. NOV. FROM SICILY

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## ABSTRACT

Field investigations in the Sicilian territory allowed study of *Pleurotus* basidiomata locally referred to as "dabs" and growing in association with *Elaeoselinum asclepium* subsp. *asclepium.* Evaluation of morphological, ecological and distribution data permitted discrimination of these populations from other closely-allied *Pleurotus* taxa growing on Umbellifers, such as *P. eryngii* var. *eryngii, P. eryngii* var. *ferulae* and *P. nebrodensis*. Therefore, it is described as a new variety, *Pleurotus eryngii* var. *elaeoselini.*

**KEY WORDS**: taxonomy, ecology, Mediterranean area, *Pleurotus*, Umbellifers.

## INTRODUCTION

The *Pleurotus eryngii* species-complex represents a typical component of the Mediterranean mycoflora. It grows in close association with Umbellifers (i.e. plants of the family Apiaceae) and produces highly prized edible mushrooms.

Despite the obvious economic importance, taxonomy within this group is still unclear and the relationships among different host-specific taxa are ambiguous since they present minute morphological differences and a partial intercompatibility which permits a limited outbreeding (Hilber 1982, Zervakis & Balis 1996).

However, especially in the geographically confined and habitat-diverse region of Sicily, ecological specialization and spatial distribution confer in a very interesting manner the delimitation of the taxa growing in Umbellifers.

The "Sicilian" *P. eryngii* species-complex comprises several distinct populations.

*Pleurotus eryngii* (DC. : Fr.) Quélet var. *eryngii* is a common taxon growing on *Eryngium campestre* L. root residues in arid pastures and it shows a fructification period from early spring to autumn.

*P. eryngii* var. *ferulae* Lanzi, growing on *Ferula communis* L. subsp. *communis* root residues in garrigues, waste lands and arid pastures, is abundant from September to the first ten days of November. During winter the basidiomata appearance is scarce but, again, it increases from the end of March until mid-June.

Both *P. eryngii* var. *eryngii* and *P. eryngii* var. *ferulae* are widespread in all the Sicilian territory mainly on calcareous soils. The former is collected from sea-level to 1300 m while the latter does not exceed altitudes of 1000 m.

*P. nebrodensis* (Inzenga) Quélet, growing on *Cachrys ferulacea* (L.) Calestani in arid pastures, is a rare taxon of the Sicilian mycological flora and its fructification period is restricted to approximately 40 days, i.e. from the middle of April to the first ten days of June. The *P. nebrodensis* growing sites are located only in the Madonie area (northern Sicily) and the Etna Mt. (eastern Sicily) on calcareous soils from 1200 to 2000 m.

Nevertheless, our attention was drawn to the locally named "dabs" or "dabbisu" mushroom, growing on *Elaeoselinum asclepium* (L.) Bertol. subsp. *asclepium* root residues.

Although this taxon is often morphologically confused with *P. nebrodensis,* anatomical, ecological and distribution characters allow its clear taxonomic delimitation from other *Pleurotus* populations associated with Umbellifers.

## MATERIALS AND METHODS

Frequent field surveys and periodical observations on *Pleurotus* taxa growing on Umbellifers were carried out in Sicily starting from 1995 until nowadays. Each taxon was evaluated as regards morphological, anatomical, distributional and ecological characters. The data obtained were also compared with the results of isozyme and molecular analysis (Zervakis and Venturella 1998, Zervakis and Venturella, unpublished data) and with the *Pleurotus* herbarium samples kept in Herbarium E. M. Fries (UPS) and Herbarium Museum Paris (PC).

More than 1500 measurements were carried out in order to calculate the spore range of each taxon investigated. Spores were taken directly from the spore prints which were obtained on glass slides. Spore sizes were measured in an aqueous solution with a micrometer (x1000). As regards the herbarium materials a piece of lamellae was taken from each sample and rehydrated in L4 or KOH 5%. Mycelium cultures were maintained on PDA (Bacto potato dextrose agar, Difco), and are stored in the Culture Collections of the authors' Institutions.

The specimens are kept in the Herbarium Mediterraneum (PAL).

## DESCRIPTION

*Pleurotus eryngii* var. *elaeoselini* Venturella, Zervakis & La Rocca, var. nov. (Fig. 1).
Etym.: from the name of the genus of the associated plant (*Elaeoselinum*).
SYN.: *Agaricus nebrodensis* Inzenga, *pro parte excl. typ.*

*Basidiomata* valida atque robusta. *Pileus* 5-14 (20) cm, convexus dein explanatus, postremo depressus, perraro reflexo-infundibuliformis. Pilei pellis haud facile separabilis, velutina atque opaca, albida, denique levis, nitida pinguisque, pallide alutacea vel incarnata, saepe areolata, praecipue ad medium, margine tenui acutaque, maxime involuta, in senectute irregulariter plana et interdum lobata. *Lamellae* spissae, arcuatae, cum abundis lamellulis, decurrentes, in juventute anastomosantes in supera parte stipitis, albae, in senectute leviter incarnatae, acie concolorata. *Stipes* 4-7,5 x 1,2-2,8 (3,6) cm, solidus, plenus, irregulariter cylindricus, ad basim attenuatus vel subradicatus, medianus vel excentricus, pileo concolor, in juventute albo-pruinatus deinde levis, glaber, in infera parte saepe leviter flavidus. *Caro* compacta, fibrosa, elastica, alba; odor saporque debiles, vulgares. *Sporae* 10-14 x 5-7 µm (Q = 1,9-2,1), hyalinae (in KOH etiamque in Melzer), leves, cylindraceae vel irregulariter ellipsoideae, pluriguttulatae, apiculo manifesto praeditae. *Basidia* 30-50 x 8-12 (14) µm, tetraspora, clavata, cum sterigmatibus 4-6 µm longis. *Cheilocystidia* 40-65 x 8-12 µm, basidiis similia, superne attenuata, interdum acute mucronata. *Pileicutis* ex hyphis cylindricis radialibus, 5-12 µm latis, constituta. Fibulae praesentes.

Locus: graegaria in herbidis locis, cum Elaeoselino asclepio consociata.

*Typus* — Madonie Mts, locality Casa Crisanti near Scillato (Palermo, Sicily), 37° 51′ 50″ N 13° 57′ 06″ E, arid pastures with *Elaeoselinum asclepium* subsp. *asclepium* on calcareous soil, 890 m, 04 May 1997, leg. G. Venturella (holotypus in PAL).

*Basidiomata* sturdy and fleshy. *Pileus* 5-14 (20 cm), convex then flat, finally depressed, seldom reflexed or funnel-shaped. Cuticle not easily detachable, velvety and opaque, whitish, then smooth, bright and greasy, with alutaceous tones or flesh-coloured; often lacerated in small appressed areolae, evident on the disk and contrasting with the white-ivory coloured flesh in the background. Margin thin, acute, deeply involute, flat in the ripe basidiomata and then irregular, sometimes also lobate. *Lamellae* thick, arcuate, mixed by many lamellulae, deeply decurrent, entirely white. Lamellae margin entire, concolorous, lightly flesh-coloured in the ripe basidiomata. The unripe basidiomata show evident anastomoses on the stipe, in some cases disappearing with the growth. *Stipe* 4-7.5 x 1.2-2.8 (3.6) cm, sturdy, filled and firm, irregularly cylindrical, attenuate at the base, sometimes radicating, central to eccentric, concolorous with the cap, lightly pruinose in the unripe basidiomata, then smooth, glabrous and yellowish at the base. *Flesh* firm and compact, fibrous, elastic, white, with a fungus-like smell and taste. *Spores* 10-14 x 5-7 µm (Q = 1.9-2.1), cylindrical to irregularly ellipsoid, smooth, hyaline, guttulate with pronounced apiculus. *Basidia,* club-shaped, 4-spored, 30-50 x 8-12 (14) µm, sterigmata 4-6 µm. *Cheilocystidia,* 8-12 x 40-65 µm, club-shaped, with thin apex sometimes showing a thin acute beak. *Epicutis* with cylindrical hyphae, 5-12 µm wide, closely appressed. Texture cutis-like. Clamp connections abundant in all parts of the basidioma. Basidiospores, basidia and trama (with occasional presence of oleiferous hyphae) hyaline in aqueous KOH and Melzer's reagent.

Culture characteristics on PDA: pigments absent, reverse colour unchanged, dikaryotic colonies mostly presenting a loose submerged and suppressed aerial mycelium, more or less zonate and radial, growth margin even and regular, colour white to cream; thin-walled hyphae, hyaline in aqueous KOH and Melzer's reagent, with abundant clamp-connections;

occasional production of microdroplets, singly on short secretory sterigmata on aerial hyphae (nematode trapping devices); optimal fungal growth 25-30 °C (2.4 mm day$^{-1}$).

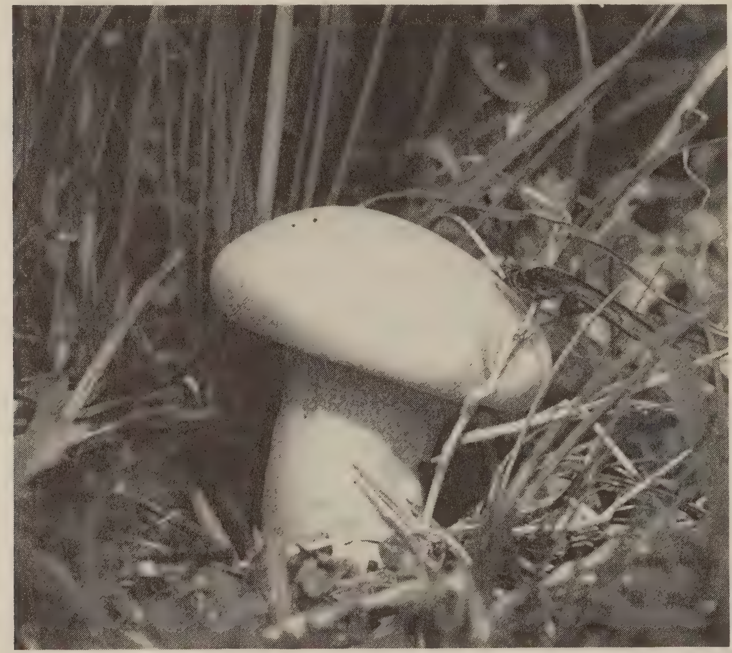

Fig. 1. Basidioma of *Pleurotus eryngii* var. *elaeoselini.*

*Specimens examined*: UPPSALA: Sicilia, *Agaricus nebrodensis*, N. Inz., F. S. p. II, Sicilia, Inzenga (Herbarium E.M. Fries, UPS!). PARIS: *Agaricus nebrodensis* Inzenga, a terra panormitana ex dono Inzenga Bot. prof. panorm. (Herbarium Mus. Paris, PC!). PALERMO: Carini, Monte Palmeto, on *Elaeoselinum asclepium* subsp. *asclepium*, calcareous soil, 500 m, 15 Mar 1994, 5 May 1995, 22 Oct 1995, 16 Apr 1996, leg. La Rocca (PAL); Godrano, Bosco della Ficuzza, Cima Cucco, on *Elaeoselinum asclepium* subsp. *asclepium*, calcareous soil, 900 m, 28 Mar 1994, 11 May 1999, leg. La Rocca (PAL); Godrano, Bosco della Ficuzza, Vallone d'Agnese, on *Elaeoselinum asclepium* subsp. *asclepium*, calcareous soil, 750 m,· 20 Oct 1995, leg. La Rocca (PAL); Bolognetta, Cozzo Pizzillo, on *Elaeoselinum asclepium* subsp. *asclepium*, calcareous soil, 500 m, 21 Apr 1996, leg. La Rocca (PAL); Palermo, Santuario Chiaranda, on *Elaeoselinum asclepium* subsp. *asclepium*, calcareous soil, 450 m, 07 Nov 1996, 16 Apr 1997, 08 Oct 1997, 27 Mar 1998, 21 Oct 1998, 04 Nov 1999, leg. La Rocca (PAL); Marineo, Piano Quadara, on *Elaeoselinum asclepium* subsp. *asclepium*, calcareous soil, 750, 10 Apr 1997, leg. La Rocca (PAL); Belmonte Mezzagno, Portella Raccuglia, on *Elaeoselinum asclepium* subsp. *asclepium*, calcareous soil, 550 m, 19 Apr 1997, 18 Oct 1998, leg. La Rocca (PAL); Collesano, Monte D'oro, *Elaeoselinum asclepium* subsp. *asclepium*, calcareous soil, 600 m, 03 May 1997, leg. Venturella (PAL); Bagheria, Monte Catalfano, on *Elaeoselinum asclepium* subsp. *asclepium*, calcareous soil, 200 m, 19 Mar 1998, leg. La Rocca (PAL); Scillato, Casa Crisanti, on *Elaeoselinum asclepium* subsp. *asclepium*, calcareous soil, 800 m, 07 May 1997, leg. Venturella (PAL); Collesano; on *Elaeoselinum asclepium* subsp. *asclepium*, calcareous soil, 470 m, 11 Apr 1996, leg. Venturella (PAL). AGRIGENTO: Sciacca, Capo San Marco, on *Elaeoselinum asclepium* subsp. *asclepium*, calcareous soil, 5 m, 13 Mar 1997, leg. La Rocca (PAL). TRAPANI: Castellammare del Golfo, Casa Iracane, on *Elaeoselinum asclepium* subsp. *asclepium*, calcareous soil, 200 m, 19 Mar 1997, leg. La Rocca (PAL); Castellammare del Golfo, Monte Erbe Bianche, on *Elaeoselinum asclepium* subsp. *asclepium*, calcareous soil, 500 m, 05 Apr 1997, leg. La Rocca (PAL). CALTANISSETTA: Resuttano, Cozzo Monaco di Mezzo, on *Elaeoselinum asclepium* subsp. *asclepium*, calcareous soil, 600 m, 20 Apr 1999, leg. Venturella (PAL); Resuttano, Cozzo Tudiotta, on *Elaeoselinum asclepium* subsp. *asclepium*, calcareous soil, 600 m, 11 Apr 1997, leg. Venturella (PAL).

## DISCUSSION

Inzenga (1863), describing *P. nebrodensis* (sub: *Agaricus nebrodensis*) reported it "in montium culminibus Siciliae, Nebrodibus magis obvia e radicibus marcescentibus Elaeoselinii Asclepii Bert, Opopanacis Chironii Koch., etc. Aprilii, Majo nive dilabente. Esculentus!".

Inzenga did not collect by himself the samples for the original description but he received them by the baron Nicolò Turrisi Colonna, a rich landowner in the Madonie area, together with information on the vernacular names of the Umbellifers on the root residues of which the fungus grows. Since in Sicily the indigenous people recognize each mushroom by calling it with the name of the plant in the vicinity of which it grows, and in the local dialect "funciu" corresponds to "mushroom", he was informed that the name "dabbisu" corresponded to *Elaeoselinum asclepium* subsp. *asclepium* and the name "basiliscu" to *Opopanax chironium* (L.) Koch and *Cachrys ferulacea*. Actually, in the Madonie area, the name "basiliscu" is only referred to *C. ferulacea*. Besides the local name "ferra" and "firrazzolu" corresponds to *Ferula communis* subsp. *communis* and *Thapsia garganica* L., respectively.

For the reasons given above, much confusion arises also on the basis of the local names of each mushroom. For example, Inzenga reported as "funciu di dabbisu" or "funciu di basiliscu" (i.e. the local names attributed to *Pleurotus* growing on *E. asclepium* subsp. *asclepium* and *C. ferulacea* respectively) the basidiomata growing on *Opopanax chironium*. He also considered as "funciu di ferra" or "funciu di firrazzolu" (i.e. the local names attributed to *Pleurotus* growing on *F. communis* subsp. *communis* and *T. garganica* respectively) the *P. nebrodensis* basidiomata growing on *Cachrys ferulacea*.

The confusion between *P. nebrodensis* and *P. eryngii* var. *elaeoselini* is due to their close morphological resemblance. In fact, macroscopic differences are limited to the heavier basidiomata of the former taxon. In contrast, they could be clearly discriminated on the basis of their microscopic characteristics (Fig. 2) and ecology.

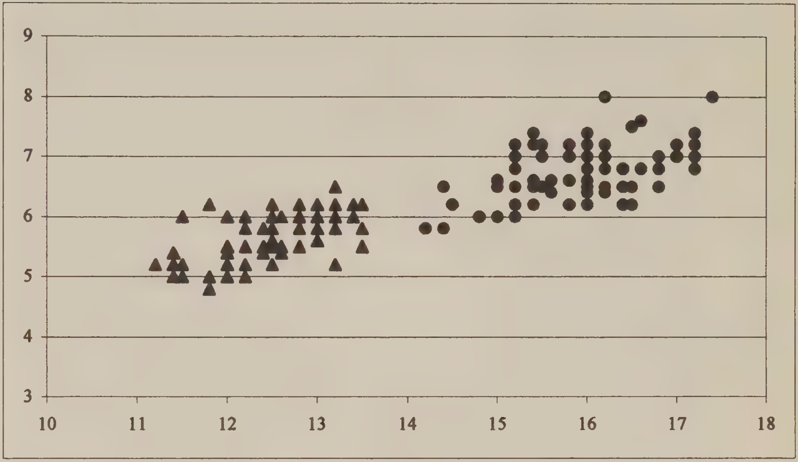

Fig. 2. Comparative diagram of *P. eryngii* var. *elaeoselini* (▲) and *P. nebrodensis* (·) showing the relative distribution of basidiospore sizes.

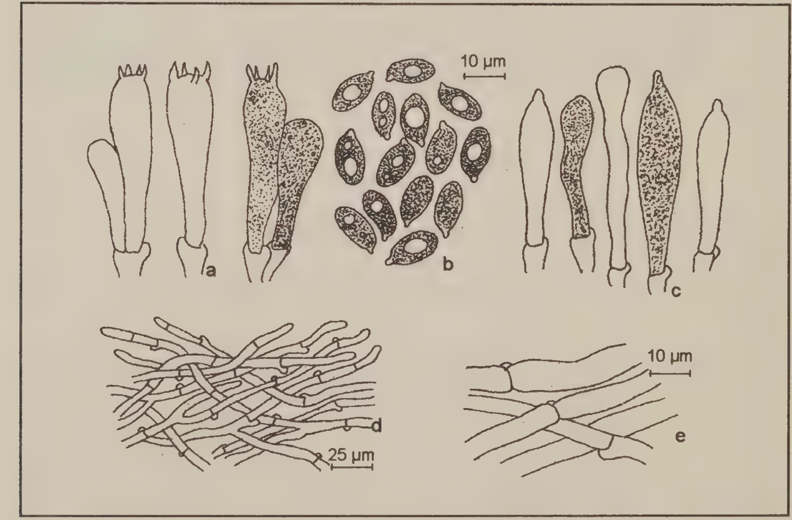

Fig.3. *Pleurotus eryngii* var. *elaeoselini* var. nov. – **a.** basidia, **b.** spores, **c.** cheilocystidia, **d.** epicutis, **e.** hymenial texture.

The basidiospore in *P. nebrodensis* are wide cylindrical (15-18 x 6-8 µm with Q = 2.1-2.5), and they are larger than the irregularly ellipsoid to cylindrical spores of *P. eryngii* var. *elaeoselini* (10-14 x 5-7 µm, Q = 1.9-2.1) (Fig. 3). The former is also characterized by cylindrical-sinuous cheilocystidia, which are more or less nodulose-gibbous and often bifid; on the other hand, the latter shows basidiole-like cheilocystidia with an attenuate apex at the top, and sometimes with an acute beak.

In Inzenga's taxonomic sense *P. nebrodensis* (sub *Agaricus nebrodensis*) and *P. eryngii* var. *elaeoselini* were considered as the same taxon. Therefore the latter is included in the herbarium samples kept in PC and UPS.

The Inzenga's *P. nebrodensis* herbarium sample kept in UPS(!) shows the typical *P. eryngii* var. *elaeoselini* microscopic characters. On the other hand, in PC(!) three *P. nebrodensis* specimens are present too and the central one was recently designated as epitype (Venturella 2000); whereas in the other two specimens the spore size corresponds to the lower limit of *P. nebrodensis* spore range, but we could not observe the cheilocystidia because the lamellae margins were eroded.

As regards ecology and distribution, *P. nebrodensis* growing on *Cachrys ferulacea* is limited to restricted areas of the Madonie Mts and the Etna Mt. in elevations ranging from 1200 to 2000 m, while *P. eryngii* var. *elaeoselini* growing on *Elaeoselinum asclepium* subsp. *asclepium* is widely distributed throughout Sicily from sea-level to 1200 m (Fig. 4).

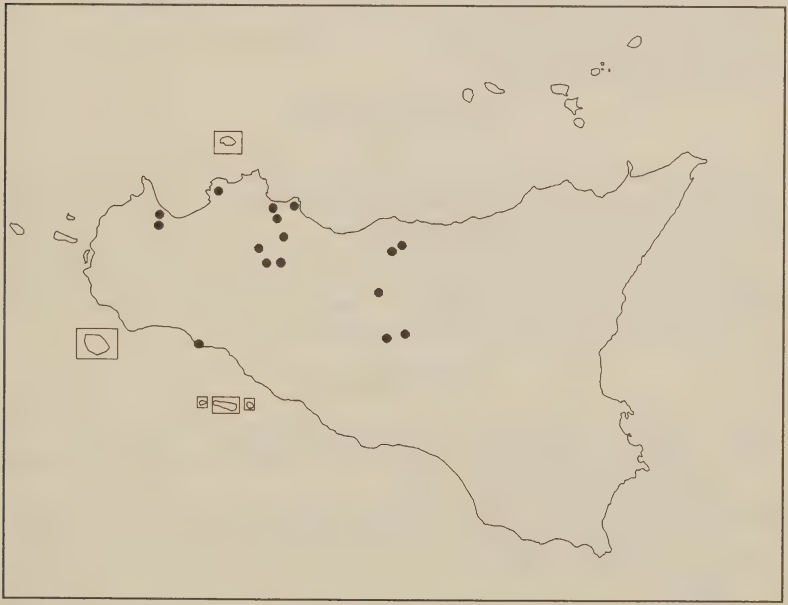

Fig. 4. Distribution of *Pleurotus eryngii* var. *elaeoselini* in Sicily.

Besides, in our field investigations we never collected *P. nebrodensis* neither on *E. asclepium* subsp. *asclepium* nor on *Opopanax chironium*.

In addition, *P. nebrodensis* produces basidiomata only from the end of April to the first ten days of June while *P. eryngii* var. *elaeoselini* basidiomata appear during the period March-May and October-November.

Besides in all the herbarium samples checked, *P. nebrodensis* is never associated with *E. asclepium* subsp. *asclepium* and *O. chironium*.

Although *P. eryngii* var. *elaeoselini* is often isolated in the same habitats as *P. eryngii* var. *ferulae*, their separation is based on macroscopic (pileus size, colour, surface and cuticle) and microscopic (spores and basidia size, cheilocystidia) morphological characters as well as on the host-plant on which they grow, i.e. *Elaeoselinum asclepium* subsp. *asclepium* and *Ferula communis* subsp. *communis* respectively.

Finally, Zervakis & Venturella (1998) evaluating data from an isozyme study conducted on *Pleurotus* taxa growing on Umbellifers and originating from several Mediterranean regions (including numerous Sicilian specimens) quoted that "although the fungal specimens collected from each host-plant presented a few morphological differences, their ecology, phenology, habit and habitat are distinct".

The results from this work suggest that the *Pleurotus* specimens collected from *E. asclepium* subsp. *asclepium* seem to be more related with specimens growing on *Ferula communis* subsp. *communis* (since both *Pleurotus* taxa are collected from similar and often identical habitats), and are clearly differentiated from *P. nebrodensis*.

The interpretation of all data support the hereby proposed description of a new *Pleurotus* taxon from Sicily.

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# NOTES ON LEPIOTA AND LEUCOAGARICUS

## Type studies on Lepiota magnispora, Lepiota barssii, and Agaricus americanus

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Type studies on some North American *Lepiota* species reveal that several well-known European names have to be changed. 1. *Lepiota magnispora* replaces *L. ventriosospora* 2. *Leucoagaricus barssii* comb. nov. is an older name for *L. pinguipes* and *L. macrorhizus*; and 3. *Leucoagaricus americanus* comb. nov. replaces *L. bresadolae*.

Keywords: Agaricaceae, *Lepiota ventriosospora, Leucoagaricus pinguipes, Leucoagaricus macrorhizus, Leucoagaricus bresadolae.*

## INTRODUCTION

The genera *Lepiota* (Pers.) Gray and *Leucoagaricus* (Locq.) ex Singer are relatively well-known in Europe (Bon, 1993; Candusso & Lanzoni, 1990). Unfortunately, the situation in North America is quite different; there there has been no modern overview of the group, and the literature on separate species is scattered with the result that these species are not well-known. Type studies on several species of *Lepiota* s.l. have indeed been published (e.g. Smith, 1966; Sundberg 1976), but proper understanding has been hampered because the species studied were not placed in a tribus or genus, nor was attention payed to infrageneric classification. Several more recent publications (e.g. Akers & Sundberg, 1997) have addressed these issues but have focused on species from Florida where the more tropical climate makes them less relevant for European mycologists. For these reasons, American species are not well known in Europe, and have not been taken into account in European overviews of the group. This paper is a step towards a synthesis of these two separate spheres of mycological knowledge and gives type studies of three American species, with a comparison of their European counterparts.

Collecting by the author in the west of the U.S.A. and in western Canada soon makes it clear that *Lepiota ventriosospora* D.A. Reid, is quite common so it seemed unlikely that it had not been described by American mycologists. A search of the literature for possible candidates led to *L. fusispora* Kauffman and *L. magnispora* Murrill; the type collection of the latter was studied.

*Leucoagaricus pinguipes* (A. Pearson) Bon was found in California and, again, a literature search revealed a possible older American candidate for that species name: *Lepiota barssii* Zeller.

Smith & Weber (1987) studied the group of species around *Lepiota americana* (Peck) Sacc. They included the European species *Leucoagaricus bresadolae* (Schulzer) Bon in their study, but concluded that the two were different. However, all published pictures of the two species show striking similarities, so again the type collection was studied, and compared with European material.

## METHODS

All material was revived in a solution of Congo Red in ammonia. The drawings of microscopical characters were made using a drawing tube.

The notation 'Spores [45, 2]' indicates that measurements were made on 45 spores from two basidiocarps. The following abbreviations are used: avl (average length), avw (average width), Q (quotient of length and width), and avQ (average quotient).

**1. Lepiota magnispora** Murrill in Mycologia 4: 237. 1912. — Holotype W.A. Murrill 704, U.S.A., Washington, around Seattle, 20-X - 1-XI-1911 (NY).

*Lepiota ventriosospora* D.A. Reid in Trans. Br. mycol. Soc. 41: 427. 1958. — *Lepiota clypeolaria* var. *pantherina* Gillet, Hyménomycètes: 62. 1874. — *Lepiota clypeolaria* var. *campanetta* Barla in Bull. Soc. mycol. Fr. 2: 116. 1886. — *Lepiota ventriosospora* var. *fulva* Bon in Bon & Chevassut in Doc. mycol. 4 (15): 27. 1974. — *Lepiota clypeolaria* f. *umbrinisquamosa* Hongo in Mem. Shiga Univ. 20: 51. 1970; *Lepiota clypeolaria* var. *umbrinosquamosa* (Hongo) Bon in Doc. mycol. 22 (88): 29. 1993. — *Lepiota ventriosospora* var. *umbrinorufescens* Bon in Doc. mycol. 22 (88): 46. 1993.

The type collection consists of two basidiocarps, in reasonable condition, with these notes: "With bay eyes and scales. Found before. Always about same size and appearance. As expands to plane, eye fades". The following microscopical characters were observed (Fig. 1).

Spores [35, 2] 15.2-20.7 x 4.5-5.8 µm, avl = 16.9 ±1.19 µm, avw = 5.1 ±0.34 µm, Q = 2.9-3.8, avQ = 3.3 ±0.27, with the same shape as some *Boletus* spores, i.e. with straight abaxial side, convex adaxial side and suprahilar depression ("penguin-shaped"), congophilous, and dextrinoid. Basidia 30-37 x 10-14 µm, 4-spored. Lamella edge not yielding any cystidia. Pleurocystidia absent. Pileus covering with long elements, c. 175-255 x 10-15 µm, with rounded apex, with pale brown intracellular pigment, some short clavate elements at base present as well. Clamp-connections present.

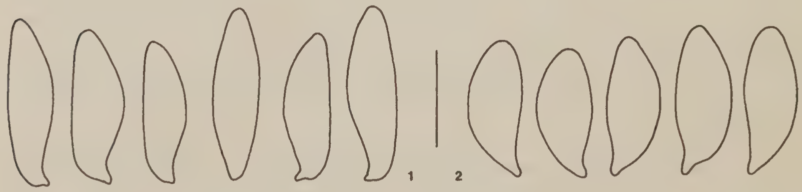

Fig. 1. *Lepiota magnispora* — Spores (from holotype).
Fig. 2. *Lepiota clypeolaria* — Spores (from O.K. Miller 8085, VPI).
Scale bar is 10 µm.

The shape and size of the spores, the presence of long and short-clavate elements in the pileus covering, and the notes accompanying the material, clearly show that this species is identical to *L. ventriosospora* D.A. Reid (1958). *Lepiota magnispora* was published in 1912, and has priority over *L. ventriosospora.*

Cheilocystidia could not be observed.

Smith (1966) also studied the type material of *L. magnispora*, and considered *L. magnispora* a synonym of *L. clypeolaria* (Bull.: Fr.) P. Kumm. Unfortunately, the drawing of the spores she gave does not reveal any details of their form, but spore sizes (viz. 14-20 x 4-5 µm), are congruent with the present findings.

*Lepiota fusispora*, described by Kauffman (1924) (non *L. fusispora* Henn., 1897) is, judging from his description and the description of the type material (Smith, 1966), also a synonym of this same species. *Lepiota fusispora* Henn. is, in spite of the similar spore size (viz. 15-18 x 4-5 µm), a different species, with a radially striate pileus, and a glabrous yellow stipe, provided with an annulus.

*Lepiota magnispora* (and *L. ventriosospora* for that matter) has not, or has hardly, been recognized in North America. However, in most cases the species depicted as *L. clypeolaria* in popular guide books is *L. magnispora* (e.g. Arora, 1986: 309; Lincoff, 1981: pl. 176; Miller, 1985: pl. 25). Peck (1884) used the name *L. metulispora* (Berk. & Broome) Sacc. for this species and this name was also in use in Europe until Reid (1958) realized that the temperate species was different from the tropical species *L. metulispora*. Both *L. clypeolaria* and *L. magnispora* occur throughout the United States but *L. magnispora* is the more common of the two.

Horak (1968) described the microscopical characters of the holotype of *Agaricus colubrinus* Pers., a synonym of *L. clypeolaria*. The spores of *L. clypeolaria* have a slightly convex adaxial and abaxial side, and a shallow suprahilar depression may be present, giving the spores an amygdaliform-fusiform appearance. The spore size is in general smaller in *L. clypeolaria* than in *L. magnispora*, and the spores are relatively broader in *L. clypeolaria*:

> *L. clypeolaria* in side-view 11.0-18.5 x (4.0-)4.5-6.0(-6.5) µm, avl x avw = 12.6-15.2 x 4.8-5.7 µm, Q = 2.1-3.4, avQ = 2.4-2.8 (based on measurements on 27 collections from Europe and 1 from Maryland, U.S.A. (Fig. 2).
>
> *Lepiota magnispora* in side-view (12.0-)13.5-25.0 x 4.0-6.0 µm, avl x avw = 15.0-21.1 x 4.7-5.45 µm, Q = (2.6-)2.7-4.85(-5.0), avQ = 3.0-4.25, (based on measurements on 50 collections from Europe, 7 from U.S.A. & Canada, 1 from China).

Besides these distinct differences in spore size and shape, the two species can also be distinguished in the field; *L. clypeolaria* has more subdued colours, without a distinct 'eye' to use Murrill's word, and the veil on the stipe is less ragged than in *L. magnispora*. For a comparison of the two species, see the pictures in Breitenbach & Kränzlin (1995: pl. 220 for *L. clypeolaria*, and pl. 235 for *L. magnispora* (as *L. ventriosospora*)).

**2. Leucoagaricus barssii** (Zeller) Vellinga, comb. nov. — Holotype S.M. Zeller 5765, U.S.A., Oregon, Yamhill Co., Newberg, Chehalem Mountain, 27-IX-1933 (NY).

*Lepiota barssii* Zeller in Mycologia 26: 211. 1934 (basionym). — *Lepiota pinguipes* A. Pearson in Trans. Br. mycol. Soc. 35: 97. 1952; *Leucoagaricus macrorhizus* var. *pinguipes* (A. Pearson) Alessio in Micol. ital. 17(2): 8. 1988 (not valid); *Leucoagaricus pinguipes* (A. Pearson) Bon in Doc. mycol. 11 (43): 54. 1981. — *Leucocoprinus macrorhizus* Locq. in Bull. mens. Soc. linn. Lyon 12: 80. 1943 (not valid, no Latin diagn.); *Leucoagaricus macrorhizus* (Locq.) Singer in Sydowia 2: 35. 1948 (not valid); *Lepiota macrorhiza* (Locq.) Kühner & Romagn., Fl. anal. Champ. sup.: 406. 1953 (not valid); *Leucoagaricus* *macrorhizus* (Locq. ex) E. Horak, Syn. Gen. Agar.: 344. 1968; *Leucocoprinus macrorhizus* (Locq. ex E. Horak) D.A. Reid in Mycol. Research 93: 421. 1989. — *Leucoagaricus macrorhizus* var. *pseudocinerascens* Bon in Doc. mycol. 20 (78): 58. 1990; *Leucoagaricus pseudocinerascens* (Bon) Bon in Doc. mycol. 23 (89): 61. 1993.

The type collection of *Lepiota barssii* consists of ten basidiocarps in good condition with the following microscopical characters (Fig. 3).

Spores [36, 2] 7.6-9.1 x 4.6-5.7 µm, avl = 8.4 ±0.47 µm, avw = 5.2 ±0.31 µm, Q = 1.45-1.8, avQ = 1.62 ±0.08, ellipsoid to oblong, some with straighter adaxial side than abaxial side, without germ pore, though in some spores the apical wall is slightly thinner than in other places, dextrinoid, and metachromatic in Cresyl Blue, congophilous. Basidia 20-28 x 7.0-8.5 µm, 4-spored. Lamella edge sterile; cheilocystidia 23-48 x 9-12 µm, narrowly clavate, cylindrical with narrowed pedicel ('hippopotamus-shaped'), colourless. Some pleurocystidia, close to lamella edge, present, similar in shape and size to cheilocystidia. Pileus scales made up of radially arranged, adnate cylindrical hyphae, 8.0-15 µm in diam., slightly thick-walled, with greyish contents. Clamp-connections not observed.

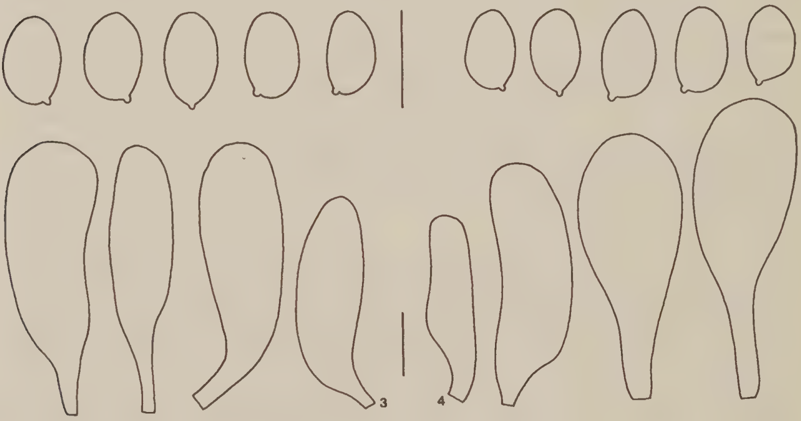

Fig. 3. *Leucoagaricus barssii* — Spores, cheilocystidia (from holotype).
Fig. 4. *Leucoagaricus pinguipes* — Spores, cheilocystidia (from holotype).
Scale bars are 10 µm.

A second collection of this species, (U.S.A., Oregon, Corvallis, Sept. 1933 (NY)) labeled co-type, was also examined, and yielded essentially the same characters as the holotype, though there is a slight variation in spore and cystidia sizes.

All characters, macroscopical and microscopical, and also the habitat, are similar to those of *Leucoagaricus pinguipes* (A. Pearson) Bon and *L. macrorhizus* (Locq. ex) E. Horak.

The type collection of *Lepiota pinguipes*, preserved at K (Braunton Burrows, N. Devon, Oct. 1949, leg. Elliston Wright) has these macroscopical characters: a fusiform thick stipe, with ring, halfway; pileus is short-fibrillose, radially squamulose, lamellae are cream coloured. Dr. C. Bas has made the following annotations on this collection (Fig. 4):

Spores 7.3-9.7 x 4.9-6.0 µm, without germ pore, dextrinoid, with metachromatic endospore in Cresyl Blue; basidia 23-32 x 7-10 µm, 4-spored; cheilocystidia 20-40 x (6-)8-13(-17-23) µm, clavate, subtriform, clavate-cylindrical, with narrow pedicel, thin-walled and colourless; pleurocystidia 38-54 x 11-16 µm, scarce, difficult to see; pileus covering with 5.0-16 µm wide hyphae; clamp-connections absent.

Horak (1968) described a lectotype for *L. macrorhizus*, in his treatise on the type species of genera of the Agaricales.

All three descriptions, viz. of *L. barssii*, *L. pinguipes*, and *L. macrorhizus*, come very close; the only difference being that Horak mentioned an indistinct germ pore to be present in some spores of *L. macrorhizus*, and pleurocystidia to be absent. A germ pore was not observed in any of the examined collections (neither the type collections, nor modern collections from both Europe and California, U.S.A.), though both light microscopy and Scanning Electron Microscopy were employed. However, some spores of the type collection of *L. barssii* do show a slightly thinner wall at the apex.

The synonymy of *L. pinguipes* and *L. macrorhizus* has been widely disputed. For instance, Bon (1993) recognized both species, and even a third, *L. pseudocinerascens* (Bon) Bon. Reid (1998) gave an elaborate overview of the literature on *L. pinguipes* and *L. macrorhizus*, covering the nomenclatorial issues involved (the name 'macrorhizus' was only validly published in 1968), and the differences between the two taxa. His conclusion, that differentiation between the two taxa on species level is not possible, is supported by the present author's observations.

In the Dutch material, the characteristic appearance of *L. macrorhizus* is an artefact of aging. This is demonstrated by an abundant bacterial flora on the pileus covering showing that specimens of this species have persisted for a long time, an unusual trait in the genus *Leucoagaricus*. Such specimens are large, have a brownish, squamulose pileus covering, and their spores are slightly longer than those of young specimens. Young and fresh specimens fit the description of *L. pinguipes* very well, and have a predominantly grey-fibrillose pileus.

Support for synonymy of *L. barssii* with *L. pinguipes* comes from molecular data; a collection of *L. barssii* from California with the same macro- and micro-scopical characters as a recent collection of *L. pinguipes* from the Netherlands, was compared with the latter and the two were found to have the same sequences of the Internal Transcribed Spacers (ITS) 1 and 2 of nuclear ribosomal DNA (sequences deposited in Genbank under accession numbers AF295931 and AF295930 resp.).

Reid (1998) did not mention the American species *L. barssii*.

*Leucoagaricus barssii* is one of the few species within the genus to possess pleurocystidia, albeit only close to the lamella edge. Other species with pleurocystidia belong to the group of *L. americanus*, e.g. *Lepiota besseyi* H.V. Sm. & N.S. Weber.

*Leucoagaricus barssii* is reported to grow in arable fields (Zeller, 1934; Horak, 1968), perhaps an indication that it was introduced to North America from Europe.

3. **Leucoagaricus americanus** (Peck) Vellinga, comb. nov. — Holotype G.W. Clinton, U.S.A.., New York, Buffalo, without date, presumably 1869. (NYS).

*Agaricus americanus* Peck in Rep. N.Y. St. Mus. nat. Hist. 23: 71. ('1870') 1872 (basionym); *Lepiota americana* (Peck) Sacc., Syll. Fung. 5: 43. 1887; *Leucocoprinus americanus* (Peck) Redhead in J.W. Groves, Edible pois. Mushr. Canada, Ed. 2: 323. 1979. — *Agaricus cupreus* Schulzer in Verh. k.k. zool.-bot. Ges. Wien 27: 112. 1877, non *A. cupreus* Secr., 1833 (not valid), nec *A. cupreus* (Krombh.) Krombh., 1846 (= *Russula cuprea*); *Lepiota bresadolae*

Schulzer in Hedwigia 24: 132. 1885, non *L. bresadolae* Henn., 1890; *Leucocoprinus bresadolae* (Schulzer) Wasser in Nov. Sist. vyssh. niz. Rast. 1977: 221. ('1977') 1978; *Leucocoprinus bresadolae* (Schulzer) M.M. Moser, Röhrlinge Blätterpilze, 4. Aufl.: 248. 1978 (superfluous comb.); *Leucoagaricus bresadolae* (Schulzer) Bon in Doc. mycol. 7 (27-28): 15. 1977.

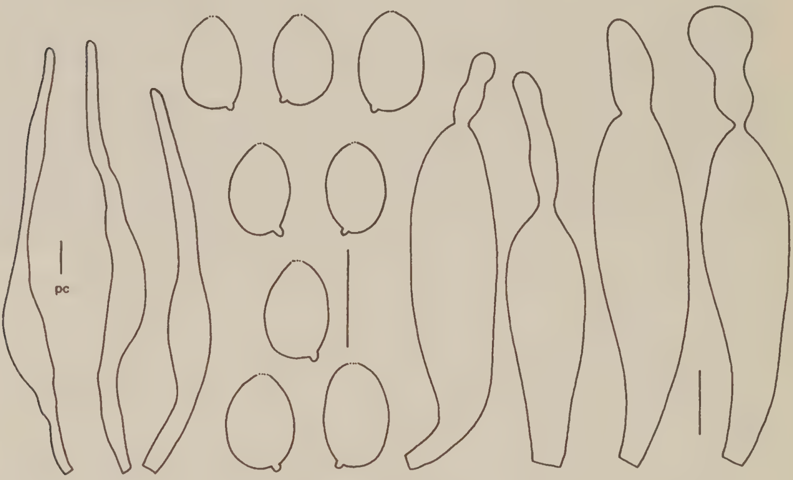

Fig. 5. *Leucoagaricus americanus* — Spores, cheilocystidia, elements of pileus covering (pc) (from holotype). Scale bars are 10 µm.

The type collection is in excellent condition, remarkably so considering its age. It consists of six specimens, one showing signs of former damage by insects; two uninfested specimens yield the following microscopical data (Fig. 5).

Spores [41, 2] 8.4-10.4 x 5.8-7.2 µm, avl = 9.2 ±0.56 µm, avw = 6.4 ±0.33 µm, Q = 1.3-1.65, avQ = 1.44 ±0.08, in side-view ellipsoid with in many cases more convex abaxial side than adaxial side, some slightly amygdaloid, in frontal view elliposid, with thick wall, with germ pore, congophilous, dextrinoid, metachromatic in Cresyl blue, cyanophilous, often pale green-yellow in ammonia. Basidia 21-26 x 7.5-10 µm, 4-spored, a very few 2-spored. Lamella edge sterile; cheilocystidia 36-65 x 12-19 µm, narrowly clavate, clavate, clavate-fusiform, rarely without, often with moniliform or cylindrical, sometimes capitulate, apical excrescence, 2.0-50 x 2.0-5.0 µm, with up to 10 µm wide capitulum, with green-brown contents in ammonia. Pleurocystidia absent. Pileus covering squamules made up of tufts of cystidioid elements; those elements very variable, in some squamules very dark and relatively short, in others pale, without parietal pigment and with long slender apical excrescence; excrescences often combined together; pigment incrusting in lower hyphae, parietal in 'pileocystidia' and also intracellular and soluble in ammonia. Stipitipellis a cutis of very narrow cylindrical hyphae, with brown intracellular pigment; caulocystidia present, some similar to cheilocystidia, but most narrowly clavate-fusiform with long slender excrescences, with brownish contents. Clamp-connections not observed.

Unfortunately, a type collection of *Leucoagaricus bresadolae* does not exist. The data on the holotype of *Agaricus americanus* are compared with those of modern collections and descriptions of *L. bresadolae.*

Reid (1990) gave a thorough overview of the European species that become green in reaction with ammonia vapour but, surprisingly, did not compare *L. bresadolae* with American species, like *L. americanus*. Smith & Weber (1987) on the other hand, did study material of both *L. americanus*, and *L. bresadolae* and described the discriminating character, viz. the structure of the pileus covering. They wrote that the pileus covering (as 'pilear cuticle') of young buttons of *Lepiota americana* is "approximately 300-400 µm thick, of interwoven to ascending hyphae, sometimes forming a rudimentary trichoderm with the upper cells but often the arrangement not so precise, many cells at least slightly inflated, slender hyphal tips often matted on the surface, no distinct pileocystidia observed, walls of cells in upper part of cuticle often pale brown. In age, cuticle similar, some of the large cells may be embedded pileocystidia; the layer pulling apart to form the scales. Scales that have been teased apart appear composed of cylindric, clavate, broadly ellipsoid, or fusoid-ventricose cells 77-138 µm long and up to 25 µm in diameter mixed with narrower hyphae." In contrast, *L. bresadolae*'s "pilear cuticle consists of a turf of versiform elongate pileocystidia, many of whose walls were light to dark brown at least at the base".

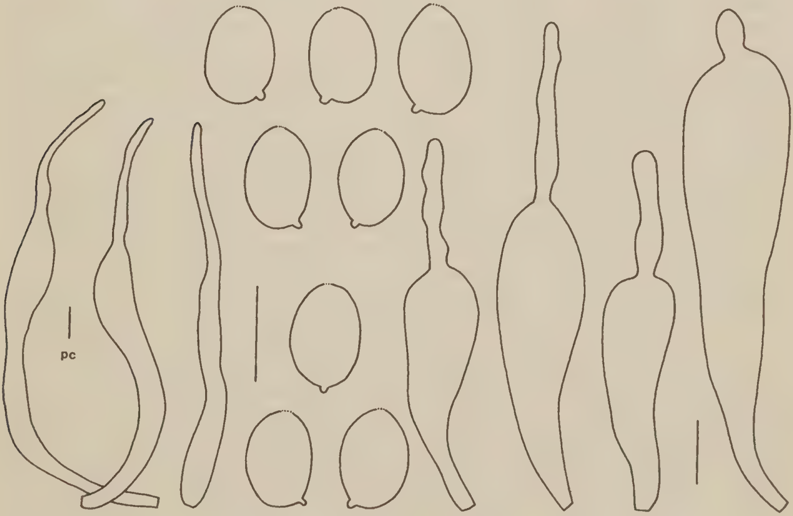

Fig. 6. *Leucoagaricus bresadolae* — Spores, cheilocystidia, elements of pileus covering (pc) (from C. Bas 7981, L). Scale bars are 10 µm.

When Reid (1990) compared *L. bresadolae* with other closely related species, such as *L. caldariorum* D.A. Reid (nom. inval.), *L. holospilotus* (Berk. & Broome) D.A. Reid, and *L. meleagris* (Sowerby) Singer, he found the most characteristic difference to be the long, sinuous, slender, tapering pilear elements (up to 260 µm long).

The structure of the pileus covering in *L. americanus* and *L. bresadolae* is very complicated, with the shape of the elements varying as well as the pigmentation. In both, *L. americanus* and *L. bresadolae*, narrowly clavate elements with a long tapering excrescence occur (these are probably the "many cells at least slightly inflated, slender hyphal tips often matted on the surface" in Smith & Weber's description (1987)). Pigment is both intracellular, and often parietal in lower part. The more parietal pigment there is, the more rigid the wall becomes, and the more easily the cells are damaged when making a slide for microscopical examination. A possible reason that Smith & Weber (1987) considered *L. bresadolae* and *L. americanus* different species, is that they had studied a great number of collections of *L. americanus*, in all stages, but only one specimen, a rather old one, of *L. bresadolae*. The original material, from which this collection of *L. bresadolae* derived, was divided and the present author was able to study another part of the very same material (Fig. 6); it is apparent that the characters fit the concept of both species extremely well. In view of the absence of any discrepancy in the descriptions and characters of the two species, *L. americanus* and *L. bresadolae* must be considered to refer to the same species. The name *Agaricus americanus* has priority as it was published 5 years before Schulzer von Muggenburg (1877) described *Agaricus cupreus*.

As with *L. barssii* and *L. pinguipes*, molecular data support this conclusion. Modern material of *L. americanus* and *L. bresadolae* (specifically including the collection from Italy, studied by Smith & Weber), have virtually identical sequences of ITS1 and ITS2 (sequences deposited in Genbank under accession numbers AF295928 and AF295929 resp.). The sequences in Genbank, under the name *L. americanus* with accession numbers U85282, U85349, and U85317 (Vilgalys & Johnson, 1998; Johnson, 1999), refer to a different, much smaller, species, which is more closely related to *L. badhamii* (Berk. & Broome) Singer.

*Leucoagaricus americanus* shows considerable variation in size and shape of the basidiocarps, and the size of the squamules on the pileus. These variants may be weather dependent. The shape of the stipe is quite constant and always fusiform. When cut, all show first a yellow discoloration, followed by red; all turn a vinaceous pink on drying. Microscopical differences between the variants could not be found.

There has been considerable confusion in Europe over the name *L. bresadolae*. First of all, the name *L. badhamii* has been used for this taxon although the spore shape in the two species is quite different: broad and ellipsoid with a germ pore in *L. bresadolae*, and distinctly amygdaloid, without a germ pore in *L. badhamii*. With further differences in discoloration of the basidiocarp on bruising and drying, and the structure of the pileus covering, it is easy to distinguish these two taxa. Demoulin (1966) has unravelled the entangled history of their confusion. Secondly, *L. bresadolae* and *L. meleagris* have been considered to be conspecific (Krieglsteiner, 1991). As pointed out by Reid (1990) the differences between those two species are to be found in the shape of the pilear elements and cystidioid elements on the stipe. Macroscopically the two are easily told apart, *L. meleagris* being the smaller with small dark brown to blackish squamules on the pileus. The North American species *Lepiota sanguiflua* Murrill is very close to *L. meleagris* and may well be identical.

## ACKNOWLEDGMENTS

The curators of NY and NYS were so kind as to provide the type collections on loan. I would like to compliment them on the excellent way they have conserved the specimens, all of which are in remarkably good condition.

Dr C. Bas contributed to this paper by sending me his notes on the type collection of *L. pinguipes*. Dr Ana Esperanza Franco Molano kindly sent me the description of her collection of *L. americanus*.

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# REDEFINING THE GENUS APLANOCHYTRIUM (PHYLUM LABYRINTHULOMYCOTA)

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ABSTRACT:

In the early 1970's, two similar genera, *Labyrinthuloides* and *Aplanochytrium*, were described within months of one another. Ironically, these two genera were originally associated with entirely different groups of fungi. *Labyrinthuloides* was recognized as having morphological similarities to the labyrinthulids (or marine slime nets), and *Aplanochytrium* was associated with the thraustochytrid fungi. However, contemporary researchers were beginning to recognize that the thraustochytrids and labyrinthulids were closely related. By the mid 1980's, biochemical and morphological evidence linking *Labyrinthuloides* and *Aplanochytrium* was mounting, and suggestions were made that these genera were identical. In 1999, Honda et al. published a molecular phylogeny of the Labyrinthulomycota, including ssurDNA sequence data from *Aplanochytrium kerguelensis*. Leander and Porter (submitted) also reported a molecular phylogeny of the group, including *Labyrinthuloides yorkensis* and *Labyrinthuloides minuta*. We have compared this sequence data from *A. kerguelensis*, *L. minuta*, and *L. yorkensis*, and together with the morphological similarities, we conclude that *Aplanochytrium* and *Labyrinthuloides* are synonymous. Following the IBC rules of nomenclature, *Aplanochytrium* is recognized as the correct name for this genus by means of precedence. Additionally, the five species of *Labyrinthuloides* are transferred to *Aplanochytrium*. We also transfer one species of *Labyrinthula*, *L. thaisi*, to *Aplanochytrium* based on morphological similarities.

KEYWORDS: *Labyrinthuloides*, stramenopile, heterokont, *Labyrinthula thaisi,* thraustochytrid

## INTRODUCTION:

In 1973, Perkins described a new genus of labyrinthulid. The genus *Labyrinthuloides* was erected based on a way of locomotion that differed dramatically from other labyrinthulids. Rather than being enrobed by the ectoplasmic net and gliding through it as in *Labyrinthula*, *Labyrinthuloides* consists of cells that can glide over a substrate by being pushed or pulled by the ectoplasmic net. Since the erection of this genus, four additional species have been described or moved to *Labyrinthuloides*. However, just months before the erection of *Labyrinthuloides*, Bahnweg and Sparrow (1972) had described a very similar genus, *Aplanochytrium*.

*Aplanochytrium* was recognized as being similar to the thraustochytrids in its thallus morphology, but was elevated to a separate genus because it makes aplanospores rather than the typical biflagellate zoospores. The aplanospores are described as "drifting" away from the parent sporangium (Ulken et al, 1985). *Aplanochytrium* has remained monotypic. Since the establishment of these two genera, there has been repeated speculation that they are indeed very closely related.

Evidence linking *Labyrinthuloides* and *Aplanochytrium* has been available for nearly 20 years. Morphologically, *Aplanochytrium kerguelensis* is most similar to (if not identical to) *Labyrinthuloides yorkensis*. These taxa are both described as having globose or spherical colorless sporangia that liberate spherical aplanospores through tears in the sporangial wall. *Labyrinthuloides yorkensis* also make zoospores, while the original generic description of *Aplanochytrium* states that only aplanospores are produced. The major morphological difference between the two taxa is in the number of aplanospores released (up to 64 in *L. yorkensis*, versus about ten in *A. kerguelensis*), and the presence of large conspicuous lipid vacuoles in the aplanospores of *A. kerguelensis* that are not seen in *L. yorkensis*.

In 1985, Ulken et al compared cell wall composition, G+C content of DNA, and nitrogen uptake ability for *Aplanochytrium, Labyrinthuloides yorkensis*, and *Labyrinthuloides minuta.* Results were so similar that they concluded, "*Aplanochytrium* and *Labyrinthuloides* seem to be very similar if not identical". Bahnweg and Jäckle (1986) examined similarities of thraustochytrid taxa using cell wall analysis, DNA base composition, and DNA/DNA hybridizations. Although they found slight differences between species of *Labyrinthuloides* and *Aplanochytrium*, the differences were usually no more than between two isolates within each genera. They concluded that these differences "indicate the presence in the oceans of a diverse flora of such organisms practically inseparable from one another on the basis of morphology." In 1989, Porter reaffirmed that *Aplanochytrium* and *Labyrinthuloides* are morphologically very similar.

In 1999, Honda et al. published a molecular phylogeny of the Labyrinthulomycota, including *Aplanochytrium kerguelensis.* Recently, Leander and Porter (submitted) also reported a molecular phylogeny of the labyrinthulids, including two species of *Labyrinthuloides, L. minuta* and *L. yorkensis*. We have compared the ssurDNA sequence data generated from these two studies in an attempt to analyze the validity of uniting *Aplanochytrium* and *Labyrinthuloides*.

Based on morphological descriptions from the original paper of *Labyrinthula thaisi*, it is obvious that this species is an aplanochytrid. This species was described before the genus *Labyrinthuloides* was established, but has never been officially transferred to *Labyrinthuloides*, as were several other taxa. Unlike other species of *Labyrinthula*, *L. thaisi* cells are not enrobed in an ectoplasmic net, and they glide on the ectoplasmic filaments, as do species of *Labyrinthuloide*s and *Aplanochytrium*. *L. thaisi* is described as making spore products that glide away from the sporangium on net elements. As this is the distinguishing character of *Aplanochytrium*, we transfer *L. thaisi* to *Aplanochytrium*.

## METHODS AND MATERIALS:

Small subunit rDNA sequence data for *Aplanochytrium kerguelensis* was obtained from Genbank. Other sequence data (for *L. minuta*, *L. yorkensis*, *Labyrinthula zosterae*, *Thraustochytrium motivum*, and *Thraustochytrium striatum*) was retained from use in a previous study (Leander and Porter, submitted). Sequences were aligned using Lineup and Pileup, available from the Genetics Computing Group, Madison, WI. Our aligned sequences (1155 bases) were analyzed by eye for base pair differences. We counted a substitution or an indel as one base difference. Distance relationships were analyzed in PAUP* (Swofford, 1998) using a Neighbor Joining algorithm.

## RESULTS:

When comparing the alignment by eye, *L. minuta* and *L. yorkensis* differed by 19 bases. *Aplanochytrium kerguelensis* differed from *L. minuta* by 17 bases, and from *L. yorkensis* by 11 bases. Thus, the sequence data from *Aplanochytrium* is more similar to each of the *Labyinthuloides* species than the *Labyrinthuloides* species are to each other (alignment available from the authors upon request). In comparison, two isolates of *Labyrinthula zosterae*, one collected from the west coast and one from the east coast of the United States, differ by 16 bases (including indels) (data not shown). Thus, the sequence similarities between *A. kerguelensis* and *L. yorkensis* are remarkable.

Our distance analysis indicates that *Aplanochytrium* nests in a monophyletic clade with the *Labyrinthuloides* species. *Aplanochytrium* is more similar in sequence to *Labyrinthula zosterae* than it is to species of *Thraustochytrium* (figure 1).

## DISCUSSION:

Keeping in mind the similarities between *Aplanochytrium* and *Labyrinthuloides* that have been previously described (Ulken, 1985, Bahnweg and Jäkle, 1986 ), as well as the sequence similarities discussed above, it is our contention that *Labyrinthuloides* is a synonym for *Aplanochytrium*. In the original description of *Labyrinthuloides* the nomenclatural type was designated by the citation of isolate 15-6-2 and the type locality was set forth. The original description of *Aplanochytrium* also includes a type slide designantion of isolate 17-4-I and type locality. Thus, *Labyrinthuloides* and *Aplanochytrium* must each be considered to have been validly published. Because *Aplanochytrium* was validly published first, the name takes priority over *Labyrinthuloides*. The name *Aplanochytrium* is the correct name following the International Code of Botanical Nomenclature (Tokyo Code), article 11.5.

Sequence similarity between the ssurDNA region of *L. yorkensis* and *A. kerguelensis* suggest that these organisms may be the same species. However, we feel that the morphological differences warrant retention of individual, yet closely related, taxa. The six recognized species of *Labyrinthuloides* are transferred to *Aplanochytrium*, as is *Labyrinthula thaisi*, based on morphological criteria discussed above.

TAXONOMY:
**Aplanochytrium** Bahnweg and Sparrow, Arch. Mikrobiol. 81. p. 46. 1972. emend. Leander and Porter. T: *A. kerguelensis* Bahnweg and Sparrow, Arch. Mikrobiol. 81. p. 46. 1972.
(=) *Labyrinthuloides* Perkins, Arch. Mikrobiol. 90. p. 5. 1973.

Sporangia are globose or subglobose, sessile or free. Walls are made of scales. Bothrosomes are present. Ectoplasmic net system is endobiotic or exobiotic, and does not enrobe the cells. Cells move independently, and may reverse direction. Applanospores always formed. Zoospores may be formed. Plasmodia and amoeboid stages may be formed.

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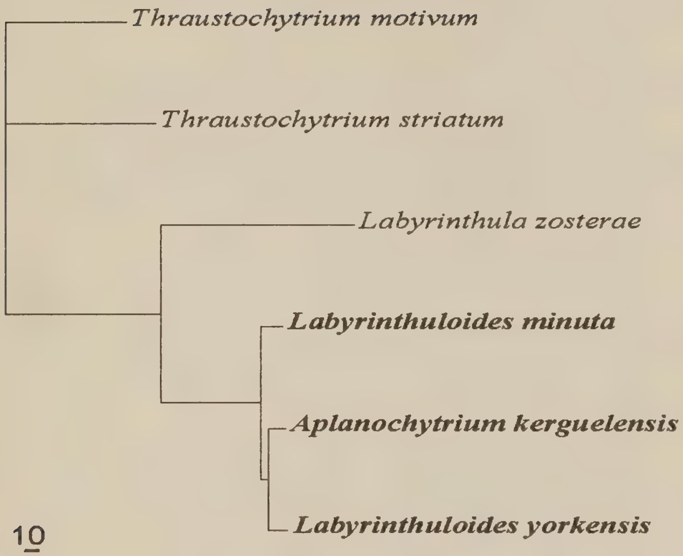
Thraustochytrium motivum
Thraustochytrium striatum
Labyrinthula zosterae
Labyrinthuloides minuta
Aplanochytrium kerguelensis
Labyrinthuloides yorkensis
10

Figure 1. Neighbor Joining tree.
Tree length = 618.
CI = 0.94. RI = 0.77.

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# CHANGBAI WOOD-ROTTING FUNGI 12. SPECIES OF HYMENOCHAETE (BASIDIOMYCOTA)

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## ABSTRACT

This paper summarizes the present knowledge of *Hymenochaete* Lév. in northern China. Nine species has been found in the area, and a key for these species is given. A new species, *H. macrospora* Y.C. Dai, is described and illustrated. It is characterized by its annual, yellowish brown basidiocarps, and large, broadly ellipsoid spores. The genus contains two additional species with large spores: *H. sphaerospora* Léger & Lanquetin and *H. senatoumbrina* Parmasto. However, the former species is perennial, and it has a grayish hymenium and globose spores. *H. senatoumbrina* has ellipsoid to subcylindric spores that are narrower than those of *H. macrospora* (5.5–7 x 3–4 μm vs. 5–7 x 3.8–5.1 μm), and its setae are shorter than those in the new species (50–70 x 6–10 μm vs. 64–125 x 6–13 μm). *H. semistupposa* Petch is newly reported from China.

Key words: Changbai Mts., China, *Hymenochaete*, *H. macrospora*, *H. semistupposa,* taxonomy, wood-rotting fungi

## INTRODUCTION

This paper is part of a series of studies devoted to the wood-rotting fungi of northeastern China, which was initiated in the Changbai Mountain in Jilin Province. For the background of the study area, see Dai (1997).

*Hymenochaete* Lév. is the second largest genus in the Hymenochaetaceae, with 260 names included in the genus, 116 of which were accepted by Léger (1998). The genus is characterized by having a smooth hymenophore, monomitic hyphal system, abundant setae, and by mostly cylindrical or ellipsoid spores. Species of the genus in northern China are not well known, and only a few publications mentioned species from China (Pilát 1940, Ryvarden *et al.* 1986, Teng 1996). During 1993–1999 extensive investigations of wood-rotting fungi in northeastern China were made, and the material of *Hymenochaete* was collected. After examining the collections at the Botanical Museum of the University of Helsinki, an undescribed species was found, and a detailed description and illustrations are provided here. Several specimens of the genus collected from the same area by other mycologists were also studied, and some collections from Beijing and Shanxi Province are included although they are outside of NE China. A key for all the species in the genus from northern China is supplied.

The microscopic method used in this study was described by Dai (1995). The terminology of the structures in *Hymenochaete* mostly follows Léger (1998). Voucher specimens collected by the senior author are deposited in HMAS (Beijing), and duplicates are preserved in Helsinki (H). To support the study, more material, especially the types of closely related species from Africa and Europe, have been examined, and these collections are cited in the specimen list.

## DESCRIPTION

***Hymenochaete macrospora*** Y.C. Dai, *spec. nov.* ( Fig. 1.)

Carpophorum annuum, resupinatum, laevigatum, ferrugineum vel umbrinum, margine lutescens. Systema hypharum monomiticum, hyphae septatae; setae adsunt. Sporae pallidae, late ellipsoideae, IKI–, CB–, 5–7 x 3.8–5.1 $\mu$m.

Holotype: China. Jilin Prov., Huinan County, Hongqi, Zhaibagou, on living tree of *Acer,* 13.VII.1993 *Dai 488* (HMAS, holotype, isotype in H)).

Basidiocarps annual, resupinate, smooth, inseparable, soft corky when fresh, without odour or taste, up to 12 cm or more in longest dimension, 10 cm wide, ca. 1 mm thick, when dry hard corky, light in weight and sometimes cracked. Hymenophore rust brown to umber brown, margin very narrow, pale yellowish brown. Context homogenous, umber brown, up to 0.7 mm thick.

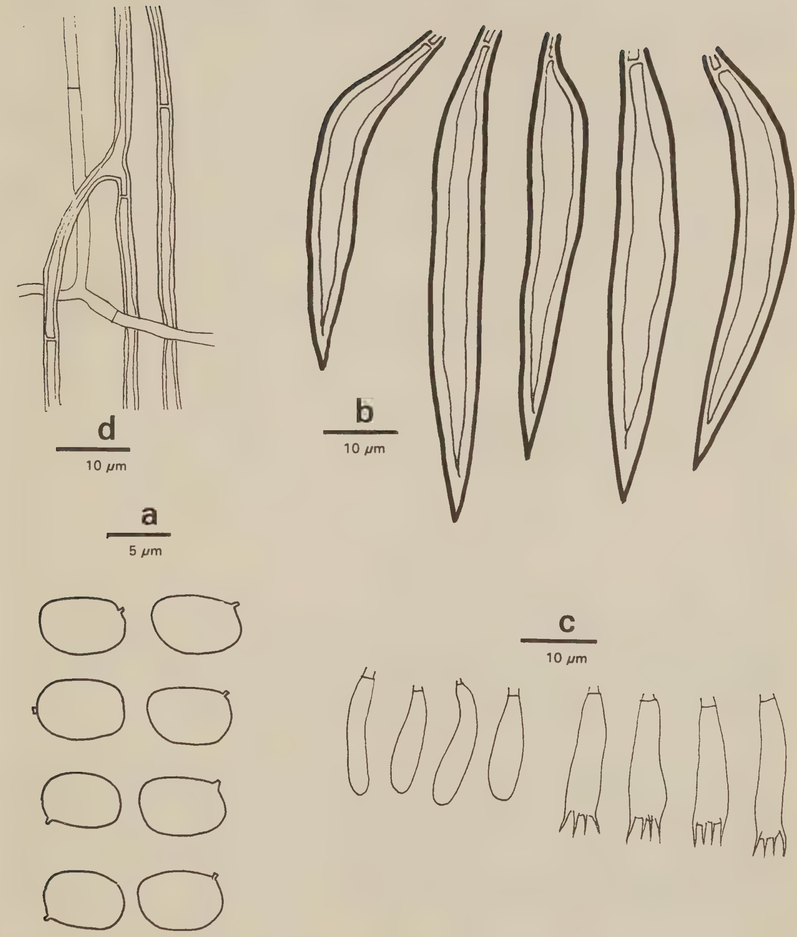

Fig. 1. Anatomical details of *H. macrospora* Y.C. Dai (drawn from the holotype). — a: Basidiospores. — b: Setae. — c: Basidia and basidioles. — d: Hyphae from subiculum.

Hyphal system monomitic; all septa without clamp connections; tissue darkening but otherwise unchanged in KOH.

*Subiculum.* — Generative hyphae varying from hyaline, thin-walled, branched, frequently septate to pale brown, thick-walled, rarely branched, flexuous, agglutinated, strongly intervowen, 2.2–4.3 $\mu$m in diam.

*Stratified subhymenium.* — Hyphae in this layer similar to those in subiculum, more frequently simple septate, more or less agglutinated, intervowen, 2–3.6 $\mu$m in diam. Setae abundant, embedded in subiculum and subhymenium, frequently penetrating beyond hymenium, subulate, dark brown, thick-walled with a wide lumen, 64–125 x 6–13 $\mu$m (n=18/2); cystidia and cystidioles not seen; basidia clavate, with four sterigmata and a simple septum at base, 13–22 x 3.8–6 $\mu$m (n=15/2), basidioles in shape similar to basidia, but smaller.

*Spores.* — Basidiospores broadly ellipsoid, hyaline, thin-walled, smooth, IKI-, CB–, (4.5–)5–7(–8) x (3.5–)3.8–5.1(–6) $\mu$m, $L = 5.89$ $\mu$m, $W = 4.47$ $\mu$m, $Q =$ 1.26–1.39 ($n$=60/2).

*Additional specimen examined (paratype).* — China. Jilin Prov., Fushong County, Shongjianghe, Shuguang, on living tree of *Ulmus,* 22.VII.1993 *Dai 656* (HMAS, H).

## DISCUSSION

The terminology of the structures in *Hymenochaete* has been discussed by Léger (1998), and the "couche sétigère" was used for the setae bearing layer. Because in some species setae are embedded in subiculum or context, and in most case setae are beyond hymenium, we use the term *stratified subhymenium* in the above description for indicating the layer between context or subiculum and hymenium.

Most species of *Hymenochaete* have small, cylindrical or ellipsoid spores, and only a few have broadly ellipsoid to globose spores (Léger 1998). *H. macrospora* is distinguished from other species in the genus by its larger and broadly ellipsoid spores. *H. sphaerospora* Léger & Lanquetin also has large spores. Type material of this tropical African species was studied. It is a perennial fungus with a gray hymenial surface and globose spores (5.5–6.2 $\mu$m in diam).

Another large spored species is *Hymenochaete senatoumbrina* Parmasto, originally described from the Russian Far East. It has ellipsoid to subcylindrical spores which are narrower than those of the new species (5.5–7 x 3–4 $\mu$m vs. 5–7 x 3.8–5.1 $\mu$m), and its setae are distinctly shorter than those in *H. macrospora* (50–70 x 6–10 $\mu$m vs. 64–125 x 6–13 $\mu$m).

Key to species of *Hymenochaete* from northern China

1. Basidiocarps effused-reflexed ---------------------------------------- 2
1. Basidiocarps strictly resupinate ---------------------------------------- 4
2. Basidiospores < 1.5 μm in width ---------------------------------------- *H. intricata* Lloyd
2. Basidiospores > 1.5 μm in width ---------------------------------------- 3
3. On several genera of angiosperms; basidiocarps soft corky when fresh, easily separable from the substrate, basidiospores cylindric to allantoid, 4.5–7 x 1.5–3.5 μm ---------------------------------------- *H. tabacina* (Sow.) Lév.
3. Mostly on *Quercus*; basidiocarps woody when fresh, hardly separable from the substrate, basidiospores ellipsoid, 4–6.5 x 2.5–3.5 μm ---------------------------------------- *H. rubiginosa* (Dicks.:Fr.) Lév.
4. Basidiospores broadly ellipsoid, 3.8–5.1 μm in width ------ *H. macrospora* Y.C. Dai
4. Basidiopsores cylindric or allantoid, 1–3 μm in width ---------------------------------------- 5
5. Basidiospores < 1.5 μm in width ---------------------------------------- *H. corrugata* (Fr.) Lév.
5. Basidiospores > 1.5 μm in width ---------------------------------------- 6
6. Basidiospores < 5 μm in length ---------------------------------------- *H. semistupposa* Petch.
6. Basidiospores > 5 μm in length ---------------------------------------- 7
7. Dendrohyphidia absent, basidiospores more or less allantoid, 5.5–6.5 μm in length ---------------------------------------- *H. cinnamomea* (Pers.:Fr.) Bres.
7. Dendrohyphidia present, basidiospores more or less cylindrical, 6–8.5 μm in length ---------------------------------------- 8
8. Mostly on *Abies*, basidiocarps bright red, setae 45–70 μm in length ---------------------------------------- *H. cruenta* (Pers.:Fr.) Donk
8. Mostly on *Rhododendron*, basidiocarps yellowish brown, setae 70–95 μm in length ---------------------------------------- *H. murashkinskyi* Pilát

*Specimens studied.* — ***Hymenochaete cinnamomea.*** China. Jilin Prov., Fushong County, Shongjianghe, on rotten wood of angiosperm, 19.VII.1993 *Dai 565.* Huinan County, Hongqi, on stump of *Syringa*, 13.X.1993 *Dai 1587.* Finland. Uusimaa, Helsinki, on fallen trunk of angiosperm 7.IX.1998 *Dai 2907*; Helsinki, *Prunus*, 22.VI.1991 *Saarenoksa 8591* (H). — ***H. corrugata.*** China. Jilin Prov., Antu County, Changbaishan Nat. Res., IX.1983 *Ryvarden 21518* (HMAS 56722). Huinan County, Hongqi, on fallen trunk of *Padus*, 12.VII.1993 *Dai 443.* — ***H. cruenta.*** China. Jilin Prov., Antu County, Changbaishan Nat. Res., on fallen trunk of *Abies*, 16.IX.1998 *Dai 2945 & Niemelä*, 17.IX.1998 *Dai 2969 & Niemelä*, 18.IX.1998 *Dai 2986 & Niemelä.* — ***H. intricata.*** China. Jilin Prov., Antu County, Changbaishan Nat. Res., IX.1983 *Ryvarden 21507 & 21526* (HMAS 56714 & 56594). — ***H. murashkinskyi***. China. Shanxi Prov., Seu Toei Chan, 14.IX.1916 *Licent 248* (PRM). Russia. Sajan Mts., *Rhododendron*, 25.VII.1927 *Murashkinsky* (PRM 686919, **holotype**). — ***H. rubiginosa.*** China. Beijing, Baihuashan, on fallen trunk of *Quercus*, 3.XI.1993 *Dai 1857.* Sweden. Halland, Särö, Västerskog, on stump of *Quercus*, 21.VIII.1996 *Dai 2255 & Niemelä.* — ***H. semistupposa***. China. Jilin Prov., Huadain County, Dongxing, on fallen trunk of *Betula*, 19.X.1993 *Dai 1737.* Wangqing County, Lanjia, on fallen trunk of *Betula*, 11.IX.1993 *Dai 1213*; on fallen trunk of *Maackia*, 13.IX.1993 *Dai 1299* **(new to China).** — ***H. sphaerospora***. Réunion.

Forest of Bébour, 12.VIII.1973 *David* (LY 7232, **holotype**). — ***H. tabacina.*** China. Jilin Prov., Antu County, Baihe, Dongfanghong, on fallen of *Tilia*, 12.VIII.1997 *Dai 2378;* Changbaishan Nat. Res., on fallen trunk of *Acer*, 29.VII.1993 *Dai 845*, 1.IX.1993 *Dai 937*; on fallen trunk of *Tilia*, 1.IX.1993 *Dai 905*. Fushong County, Shuguang, on fallen trunk of *Acer*, 20.VII.*1993 Dai 581 & 610*. Huinan County, Hongqi, on fallen branch of angiosperm, 12.VII.1993 *Dai 442*; on dead tree of angiosperm, 11.X.1993 *Dai 1534*. Wangqing County, Lanjia, on fallen trunk of *Betula*, 10.IX.1993 *Dai 1190*.

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# THE HYPHOMYCETE GENERA EXOCHALARA AND MONILOCHAETES

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## Abstract

The fungus *Monilochaetes infuscans* causes scurf disease or soil stain of sweet-potato, *Ipomoea batatas*. The disease was known from America since 1890, later also from Australia, Asia, Europe, New Zealand, and the Pacific Islands; in South Africa it was reported in 1995. Comparative morphological and cultural investigations of *M. infuscans*, the type species of the genus, and species of the related genera *Exochalara* and *Dischloridium* indicated that *D. cylindrospermum* is conspecific and *E. guadalcanalensis* congeneric with *M. infuscans*. The new combination *M. guadalcanalensis* is proposed. This arrangement results in *Monilochaetes* accommodating two species, while the type species of *Exochalara*, *E. longissima* (Grove) W. Gams & Hol.-Jech., and a subsequently described species, *E. imbricata* Hol.-Jech., are retained in *Exochalara*, a genus characterized by sympodially branching, rather pale brown, concolorous conidiophores and rather narrow, fusiform conidia. The type species of *Dischloridium*, *D. laeënse*, is quite distinct in having strongly fasciculate conidiophores and much larger conidiogenous dimensions, but other species described in that genus may also be assigned to *Monilochaetes*, but the generic limites are not yet sufficiently defined.

## INTRODUCTION

Scurf disease or soil stain of *Ipomoea batatas* (L.) Lam. (sweet-potato) affects the appearance of tubers and can also result in shrinkage of tubers because of water loss in storage (Clark & Moyer, 1988). Symptoms are restricted to the periderm and are not visible in the cortex or underlying tissues of the storage root (Clark & Moyer, 1988). The disease has been reported from localities in Australia, Asia, Europe, New Zealand, North- and South-America, the Pacific Islands and southern Africa (Anonymous, 1990; Thompson *et al.*, 1995).

Halsted (1890) was the first to ascribe scurf disease observed in New Jersey to a fungus. He provided an illustration and gave the fungus its present name. A description was published only in 1916 by Harter (1916) who based it on fresh material of the commonly observed disease. Because a diagnosis for a new species was not required for valid publication until 1908 (Art. 42.3 ICBN), the species name can properly be attributed to Halsted alone. In the description, Harter still failed to notice that conidia of this fungus form chains, an aspect later reported by Taubenhaus (1916). Harter (l.c.: 791) stated that several type specimens were deposited at the Department of Agriculture, Washington DC. (now BPI).

Subsequently, *Exochalara* W. Gams & Hol.-Jech. was described with two species, viz. *E. longissima* (Grove) W. Gams & Hol.-Jech. and *E. guadalcanalensis* (Matsush.) W. Gams & Hol.-Jech. (Gams & Holubová-Jechová, 1976); a third, *E. imbricata* Hol.-Jech., was added by Holubová-Jechová (1984). All three species colonize dead woody substrata. No teleomorph has been reported for any of the species of the two genera. Sutton (1977) introduced a further genus, *Dischloridium*, for *Chloridium laeënse* Matsush. (Matsushima, 1971). This genus was characterized by immersed stromata, composed of dark brown, thick-walled pseudoparenchyma, from which arise densely caespitose, very large, unbranched, brown (upically paler) conidiophores, bearing large ellipsoid phialidic conidia in slimy heads. Thirteen species have been added to this genus.

In characterizing the fungi that were isolated from sweet-potato, we found similarities between *Monilochaetes*, *Exochalara*, and *Dischloridium*. One species described in *Exophiala* and three in *Dischloridium* turned out to be very similar to *Monilochaetes*, while one species described in *Dischloridium* turned out to be a synonym of *M. infuscans*. These species are discussed here.

## MATERIAL AND METHODS

Isolates were cultured in the dark on oatmeal agar (OA), potato-carrot agar (PCA) and 1.5% malt extract agar (BioLab, MEA) at temperatures of 5, 15, 20, 25, and 37°C (Booth, 1971). On the tenth day after inoculation, measurements were made and features of colonies recorded. Colony colours are described according to the Methuen (M) (Kornerup & Wanscher, 1978) and Rayner (1970) (R) colour charts. Microscopic mounts used for measuring were made in clear lactic acid and lactophenol. Herbarium specimens of the relevant species were also examined.

## *MONILOCHAETES* Halsted 1890

Species forming grey to olivaceous, odourless colonies. Conidiophores subtended by swollen, darkened cells, upright, single, thick-walled, dark at the base and paler at the tip, with prominently darkened septa; intervals between these septa depending on the substrate and growth conditions. Conidia 4 µm or more wide.

***Monilochaetes infuscans*** Ellis & Halsted, in Halsted 1890, New Jersey Agric. Exp. Stn Bull. 76: 27. 1890 (Figs 17 a–e). — Figs. 1–4, 7.
= *Dischloridium cylindrospermum* Srivastava 1986, Sydowia 39: 217.
Description: Harter 1916, J. Agric. Res. 5: 791.

*Colonies* growing very slowly, on OA umbonate in the centre, iron grey (M: 29D1–2; R: 122) to pale olivaceous-grey (M: 29–30E1–F1; R: 121), with submerged edge and reverse greenish black (M: 29F5–6; R: 124). Optimum growth 7–9 mm diam and sporulation at 20°C on OA after 10 days. *Mycelium* internal on tuber of sweet potato, light brown, hyphae 2.5–4 µm wide. *Sclerotia*, 70–170 µm diam, seen on the host only, irregular in shape, black but with individual cells clearly discernable. *Conidiophores* arising from the tuber surface or superficially in culture, upright, thick- and smooth-walled, mostly single but rarely once basitonously branched, in pairs, curved or geniculate; on the host with 3–5(–8) darkened septa; in culture regularly septate at 20–25 µm intervals, subtended by 1–4 rounded, dark, thick-walled, 10–12.5 µm diam cells, then 5–7 µm wide and gradually paler towards the apex; conidiophores on the host 60–400, in culture 300–400 µm tall. *Conidiogenous cells* terminal, integrated, monophialidic, cylindrical, slightly tapering towards the 2–3 µm wide neck, with a periclinal wall thickening and a minute inward-curved collarette; on the host 12–48 µm long, in culture 50–63 × 5–6.5 µm. *Conidia* in chains, smooth-walled, cytoplasm granulose, aseptate; on the host rhomboid–ellipsoidal to obovoid; in culture ellipsoidal with slightly pointed tip, with a narrowly truncate base and a small fringe remaining after abscission; hyaline, 15–20 × 4–6 µm, mostly 19 × 5 µm.

**Material examined**:

**Cultures:** **CBS 379.77** ex *Ipomoea batatas*, New Zealand, T.W. Canter-Visscher; **PPRI 6212** (CBS 870.96), **ex-epitype** (PREM 51762); **PPRI 6216** (CBS 869.96) ex *Ipomoea batatas*, Gamtoos, Eastern Cape Province, South Africa, P.R. Ferreira.

**Herbarium specimens:** **BPI 423230**, marked "**Neotype**", ex *Ipomoea batatas*, Washington DC., Jan. 1916, L.L. Harter 1374 b; **BPI 423564** marked "Neotype?", *Ipomoea batatas*, L.L. Harter NFC 155, Jan. 1916; **BPI 423576** *Ipomoea batatas*, Raleigh, N.C., H.R. Fulton, Dec. 1912; **BPI 423601** sweet-potato, West Raleigh, N.C., H.R. Fulton, Jan. 1913; **BPI 407033, 423577 & 423578** ex rind of sweet potato, Waterford, N.J., C.A. Schwarze, Nov. 1917. **PREM 51832** ex *Ipomoea batatas*, cv. Blesbok and Bosbok, D. Nel (873 u/91), Aloe Hill Farms, Ohrigstad, Transvaal, May 1991; **PREM 51762** ex *Ipomoea batatas* cv. Blesbok, (Culture PPRI 5458), D. Nel (418T/u/94) Patatfontein Farm, Levubu, Northern Transvaal, May 1994. **PREM 57083** ex *Ipomoea batatas*, Marble Hall, Mpumalanga Province, South Africa, February 1996, I.H. Rong, **epitype** (designated here). **IMI 215291**, holotype of *Dischloridium cylindrospermum*, on dead stems of *Ipomoea fistulosa*, Gorakhpur, India, 1987.

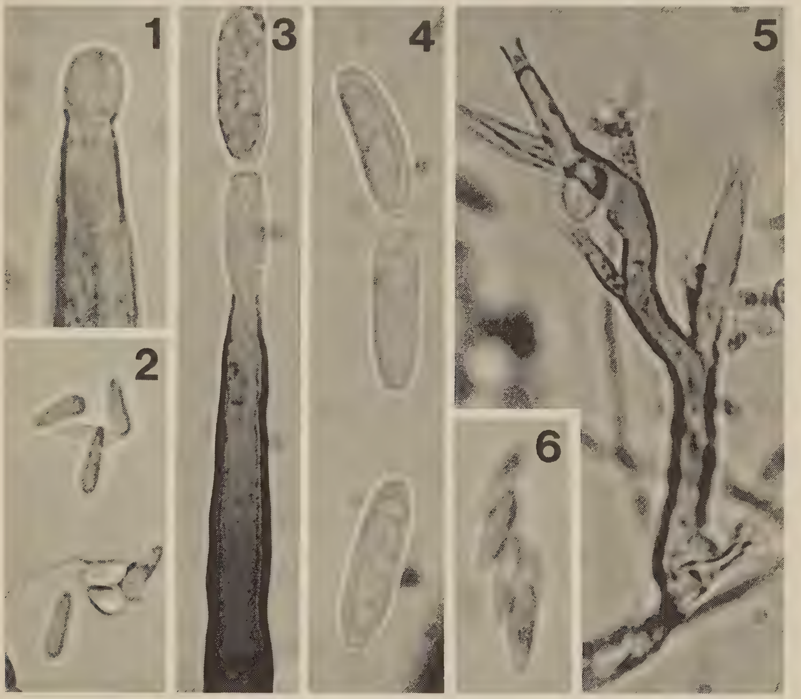
Clark & Moyer (1988) noted the formation of appressoria with a single penetration peg in *M. infuscans*. Similarly, in this study we noted cells clearly darker than the supporting hyphae and separated by a distinct septum between the cells of tubers infected by *M. infuscans*. Actual penetration of host cells was not observed but it was apparent that conidiophores originated directly from these cells.

Conidiogenous cells and conidiophores formed in culture are longer than those on the host; their dimensions vary with cultural conditions. Conidial shape, mostly considered a good taxonomic characteristic, also varied according to conditions.

None of the herbarium material examined from BPI was found to be adequately diagnostic. There is one specimen left from Harter, BPI 423230, which is obviously to be regarded as the neotype; it contains a few conidia but no intact conidiophores. To settle the identity of the fungus unequivocally we here designate an epitype, PREM 57083 (cultures PPRI 6212 = CBS 870.96). Examination of the type specimen of *Dischloridium cylindrospermum* confirmed its identity with the present species; its cylindrical conidia were described as measuring 18–22 × 4.5–5.7 µm (Srivastava, 1986), while we observed them as measuring 15–25 × 5.0–6.5 µm; the host genus, *Ipomoea*, is also identical.

***Monilochaetes guadalcanalensis*** (Matsush.) I.H. Rong & W. Gams *comb. nov.* — Fig. 8.

≡ *Catenularia guadalcanalensis* Matsush. 1971, Microfungi of the Solomon Islands and Papua-New Guinea, Kobe, p. 10 ≡ *Exochalara guadalcanalensis* (Matsush.) W. Gams & Hol.-Jech. 1976, Stud. Mycol. 13: 58.



**Figs. 1–4.** Photomicrographs of *Monilochaetes infuscans* in cultures: 1. Conidiogenous cell of PPRI 6362, with periclinal wall thickening and collarette (× 2800), 2. Obovoid conidia of CBS 379.77 on 1.5% MEA (× 550), 3. conidia formed in a chain (× 1600); 4. ellipsoidal conidia of PPRI 6362 on PCA (× 1400). **Fig. 5.** *Exochalara longissima*, CBS 622.82, on PCA, branching conidiophore and conidiogenous cells with distinct collarettes (× 1500). **Fig. 6.** *Exochalara imbricata*, PRM 829861, Holotype, on rotten wood; imbricate chain of conidia (× 3000).

*Colonies* fast growing, reaching 32–35 mm diam on OA in 10 days at 20°C, appearance woolly, mouse-grey (M: 2D2; R: 117–118), margin concolorous but submerged, reverse smoky-grey to grey-olivaceous (M:

3C2–3; R: 107). *Vegetative hyphae* brown, (1)–2.5–(4) µm wide. *Conidiophores* mostly single arising from slightly swollen, rough-walled cell or cells, 7–10 µm wide, in the substratum, dark brown and thick-walled near the base but paler and slightly rough-walled near the tip, 150–220(–400) × 4–7 µm. *Conidiogenous cell* terminal, integrated, monophialidic, cylindrical with an acute tip, 35–65 µm long, thin-walled in the upper half, ending with a slight, flaring 4 µm wide collarette and distinct periclinal wall thickening. Collarette subtly darker than the wall below. *Conidia* in chains, smooth-walled, conspicuously guttulate, ellipsoidal with a truncate base, aseptate and mostly 18–21 × 6–9 µm.

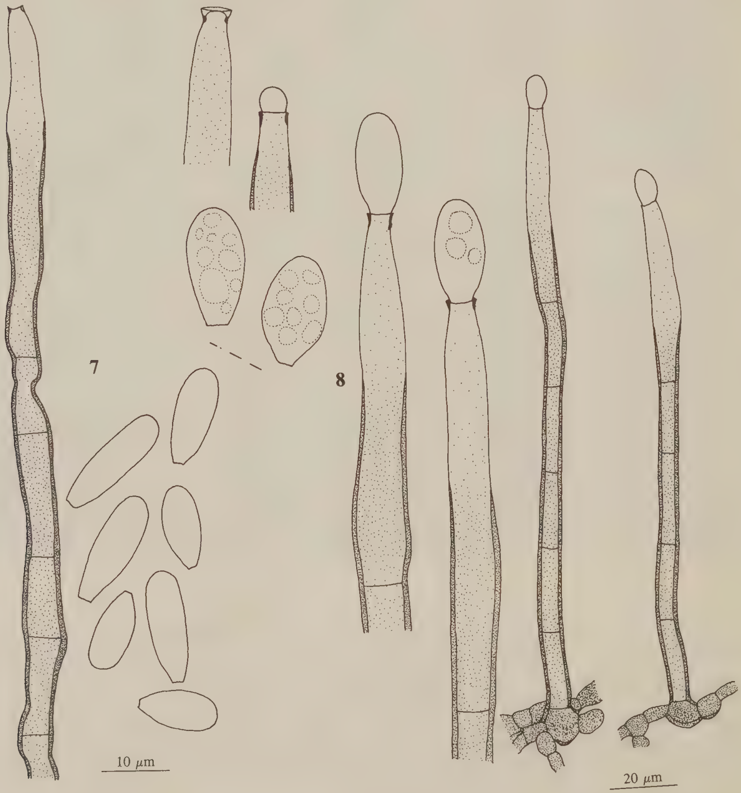


**Fig. 7.** *Monilochaetes infuscans* in culture, CBS 870.96, after 30 days on OA. **Fig. 8.** *Monilochaetes guadalcanalensis*, MFC-2875 = CBS 346.76, after 9 days on 2% MEA. Left bar applies to 7 and left part of 8.

**Material examined:**
**Culture: CBS 346.76** (MFC 2875) **ex-type**, decaying leaf of *Musa* sp., Honiara, Guadalcanal Island, 3 Jan. 1970, T. Matsushima.

*Monilochaetes infuscans* is distinct from *M. guadalcanalensis* in being pathogenic to *Ipomoea*, much slower-growing, having smooth-walled conidiophores and more narrow and more fusoid conidia. On the media tested, the only isolate of *M. infuscans* that grew at 5°C was CBS 379.77. Neither *M. infuscans* nor *M. guadalcanalensis* grows or survives 10 days' incubation at 37°C. Optimum temperature for growth and sporulation of both these species is near 20°C on OA. After 10 days' incubation at 25°C on OA, isolates of *M. infuscans* sporulated weakly whereas *M. guadalcanalensis* did not produce conidia.

## ***EXOCHALARA*** W. Gams & Hol.-Jech. 1976

Species with sympodially branching conidiophores that are rather pale and concolorous, often clustered along a hypha. Septa in conidiophores are darkened and usually located at the base of each branch of the conidiophore. Conidia less than 4 µm wide.

***Exochalara longissima*** (Grove) W. Gams & Hol.-Jech., 1976. Stud. Mycol. 13: 56. — Fig. 5.

≡ *Chalara longissima* Grove 1885, J. Bot., Lond. 1885: 12.
= *Catenularia piceae* M.B. Ellis 1976, More dematiaceous Hyphomycetes: 443.
Synonyms *fide* Gams & Holubová-Jechová (1976).

*Colonies* rather fast-growing, reaching 23–25 mm diam in 10 days on OA, with a distinct fermented fruity smell; upper surface vinaceous-buff to hazel (M: 5B–C2–3 and C4; R: 86 & 88), margin concolorous, not differentiated, reverse paler. *Hyphae* hyaline, 1–2 µm wide. *Conidiophores* abundant, commonly clustered together on the same vegetative hyphae and often once or twice sympodially branched, light brown, concolorous, 30–60 × 3.5–4 µm, including the terminal conidiogenous cell. *Conidiogenous cells* subulate, 23–27 µm long and 3.5–4 µm wide at the base, 2 µm at the tip, with pronounced periclinal wall thickening and funnel-shaped collarettes. *Conidia* adhering end to end in long chains, fusiform to clavate, 5.5–10 × 3.5–4 µm, both ends mostly truncate and with a distinct scar.

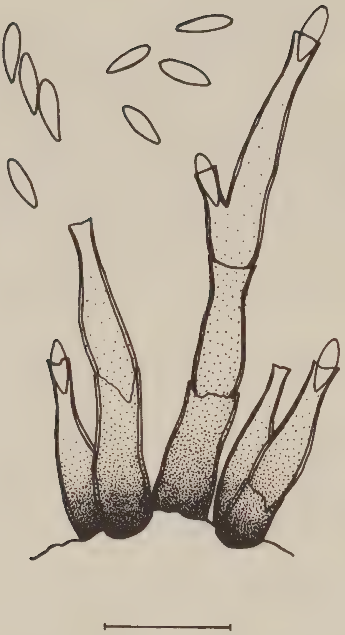


Fig. 9. *Exochalara imbricata*, PRM 829861, Holotype. Conidiophores and conidia. Bar = 10 µm.

**Material examined:**

**Cultures:** **CBS 980.73** ex decaying wood, Netherlands; Baarn, Estate Pijnenburg, W. Gams, Aug. 1972; **CBS 276.80** ex *Bulgaria inquinans*, Germany; Eifel, near Gerolstein, W. Gams, 1979; **CBS 393.82** ex decomposing needle of *Abies alba*, France; Villeurbanne, F. Gourbière, No. A141, 1982; **CBS 622.82** ex *Bulgaria inquinans*, Ardennes, Forêt de Resteigne, Belgium, W. Gams, Oct. 1982.

**Herbarium specimens:** **IMI 18047**, slide from Type Collection, dead wood, Birmingham, England, Sep. 1884; **IMI 32266** *Pteridium aquilinum*, Surrey, England, S.J. Hughes, Nov. 1948; **IMI 167413** Type of *Catenularia piceae*, *Picea* sp., Hartland Forest, N. Devon, England, M.B. Ellis, May 1972. — **PRM 793986** *Fagus sylvatica,* nr. Černý potok river, Jizerské hory Mts., Czech Republic, Eastern Bohemia, Aug. 1965; **PRM 794078** *Betula verrucosa*, Kačín nr. Lamač, Slovak Republic, Sep. 1975; **PRM 79062** *Picea abies*, Mt. Královsky kámen nr. Kašperské Hory, Czech Republic, Southern

Bohemia, Oct. 1975; **PRM 869468** stems of *Alnus glutinosa*, Revúca, Slovak Republic, Sep. 1986, all collected by V. Holubová-Jechová.

Previous descriptions of the fungus: in *Catenularia* (Ellis, 1976), referring it possibly to *Chalara* (Nag Raj & Kendrick, 1975), and in *Exochalara* (Gams & Holubová-Jechová, 1976; Holubová-Jechová, 1984).

Conidiophores formed on host material often arise singly and proliferate percurrently. They are on average longer (more than 100 μm) than those formed in culture. Conidiophores formed in culture, especially on OA, tend to be once or twice (sometimes more) sympodially branched, and clustered together on the same side of a vegetative hypha. Formation of conidia in chains is more prominent on natural substrata and on media poor in nutrients such as PCA.

***Exochalara imbricata*** Hol.-Jech. 1984. Folia Geobot. Phytotaxon. 19: 418. — Figs. 6, 9.

No cultures are available. The species is characterized by its erect, clustered conidiophores with integrated, phialidic conidiogenous cells that end in a funnel-shaped collarette. Conidia are fusiform, aseptate 4–5 × 1–1.5 μm and adhere in imbricate chains. These characteristics are clearly visible on the type material.

**Material examined:**
**PRM 829861** Holotype, on rotten wood of *Fagus sylvatica*, Moravskoslezske Beskydy Mts., Mionsi forest near Jablunkov, Czech Republic, Moravia, V. Holubová-Jechová, 28 July 1982.

*Exochalara longissima* is distinct from *E. imbricata* in that it forms much larger conidia that are not retained in imbricate chains. In addition, *E. longissima* can grow at 5°C and survive 10 days' incubation at 37°C. Its optimum temperature for sporulation seems to be between 15 and 20°C, but radial growth is best at a temperature closer to 25°C.

## *DISCHLORIDIUM* B. Sutton 1977

We have not studied material of ***D. laeënse*** (Matsush.) B. Sutton, which was originally isolated from leaf litter in Lae, Papua-New Guinea. Judging from the protologue, this species is obviously quite distinct from *M. infuscans* by having fasciculate pigmented conidiophores and altogether much larger structures, with conidia reported to measure 17–26 × 8–12 μm (Matsushima, 1971). Several other species have been described in this genus. Some of them have unpigmented conidiophores or septate conidia, rendering the genus heterogeneous (Holubová-Jechová, 1990). For comparison with *M. infuscans*, we consider only species that are described as having pigmented conidiophores and non-septate conidia, without providing a comprehensive review of the material. No revision of the whole genus is yet attempted.

***Dischloridium tenuisporum*** Hol.-Jech. (Holubová-Jechová, 1987), collected on leaf litter of *Clusia rosea* in Cuba, has fasciculate conidiophores and ellipsoidal to elongate-ellipsoidal conidia, 10–18 × 3.5–5 µm. – ***Dischloridium regenerans*** D.J. Bhat & B. Kendrick (1993), collected on dead twigs of a strangler fig in India, has single, very large conidiophores and ellipsoid conidia, 25–38 × 12–16 µm; otherwise it resembles *M. guadalcanalensis*. – ***Dischloridium cylindrospermum*** Srivastava (1987), was found to be identical with *M. infuscans*. – ***Dischloridium basicurvatum*** Matsush. (Matsushima, 1995), found on a decaying petiole of a palm in Amazonian Peru, has basally curved conidiophores and oblong-ellipsoid conidia, 9–25 × 3.5–6(–7) µm.

## KEY TO THE SPECIES OF *MONILOCHAETES*, *EXOCHALARA* AND PART OF *DISCHLORIDIUM*

**1a.** Conidia ellipsoidal, at least 17–26 × 8–12 µm . . . . . . . . . . . . . . . **2**
**b.** Conidia ellipsoidal or with somewhat pointed tip or fusiform, smaller **3**

**2a.** Conidiophores strongly fasciculate; conidia 17–26 × 8–12 µm . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . ***D. laeënse***
**b.** Conidiophores not fasciculate; conidia 25–38 × 12–16 µm . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . ***D. regenerans***

**3a.** Conidiophores mostly single, subtended by one or more enlarged, darkened cells; paler towards the tip; conidia at least 15 × 4 µm, ellipsoidal . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . **4**
**b.** Conidiophores often clustered and/or conidia smaller . . . . . . . . . . **6**

**4a.** Conidiophores with a basal curvature; conidia 9–25 × 3.5–6(–7) µm . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . ***D. basicurvatum***
**b.** Conidiophores with straight base . . . . . . . . . . . . . . . . . . . . . . . . . **5**

**5a.** Conidia rhomboid–ellipsoidal to obovoid on the host, in culture ellipsoidal with a truncate base and 15–20 × 4–6 µm; colonies slow-growing (7–9 mm diam in 10 days on OA at 20°C) . . . . . . . . . ***M. infuscans***

**b.** Conidia in culture ellipsoidal with a truncate base, 18–21 × 6–9 µm; colonies fast-growing (32–35 mm diam in 10 days on OA at 20°C) . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . ***M. guadalcanalensis***

**6a.** Conidiophores arising in fascicles from host substratum, brown, paler upwards; conidia 10–18 × 3.5–5 µm . . . . . . . . . . . . ***D. tenuisporum***

**b.** Conidiophores clustered along a vegetative hypha, paler brown, concolorous, with no enlarged cells at the base; conidia considerably smaller, ± fusiform . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 7

**7a.** Conidia fusiform, 4–5 × 1–1.5 µm, adhering in imbricate chains . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . ***E. imbricata***

**b.** Conidia fusiform to clavate, 5.5–10 × 3.5–4 µm, one or both ends truncate, chains adhering end-to-end . . . . . . . . . . . . . ***E. longissima***

## DISCUSSION

The genus *Monilochaetes* is distinct from *Exochalara* and probably also from *Dischloridium*. *Monilochaetes* and *Exochalara* are similar in that both include species with aseptate hyaline conidia in chains. The type species of *Dischloridium* has densely caespitose conidiophores arising from an immersed pseudoparenchymatous stroma inside the plant substratum; its conidiogenous structures are much larger than those of *M. infuscans*. Other species described in the genus appear, however, closer to *Monilochaetes*. The delimitation of the genus is not yet settled and we therefore leave the species in the genus as originally designated. As no teleomorph is known in any of the species considered (apart from a possibly chaetosphaeriaceous one mentioned for *D. laeënse* by Kirk, 1986), their insertion in the system of ascomycetes will have to rely on molecular findings.

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# TAXONOMY OF THE RUSSULA XERAMPELINA GROUP
# I. MORPHOMETRIC STUDY OF THE RUSSULA XERAMPELINA GROUP IN SLOVAKIA

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## Abstract

Morphometric analyses were performed on 67 fruitbodies of *Russula* sect. *Xerampelinae* with prevailingly purple, purple-brown and red-brown pileus cuticle from coniferous forests collected in selected localities in Slovakia. Multivariate methods used include principal components analysis and canonical discriminant analysis. Sixty one characters were measured or scored for each fruitbody. Many of traditionally used characters were precisely defined or modified, especially micromorphological characters of spores and pileus epicutis. The observed material is composed from three morphologically and ecologically defined groups. The group of fruitbodies with purple-brown or red-brown cuticle of pileus is labelled as group A and the other two groups with purple cuticle of pileus as group B. We suggest to classify groups A and B as species. Variability observed within fruitbodies of group B from Záhorská nížina lowland and from other localities is regarded as infraspecific, and we suggest to range it on the level of variety.

Key words: Russulales, *Russula xerampelina*, *Russula erythropoda*, multivariate morphometrics, Slovakia.

## Introduction

*Russula erythropoda* Peltereau and *R. xerampelina* (Schaeff.) Fr. are species of *Russula* sect. *Xerampelinae* (Singer) Jul. Schäff. (the often used name for this section, "*Viridantinae* Romagnesi", is invalid). Both have a prevailingly purple, purple-brown and red-brown colour of pileus cuticle, and are associated with coniferous trees. *R. xerampelina*, the type species of this section, was described by SCHAEFFER (1774) as *Agaricus xerampelinus* and FRIES (1838) made the corresponding combination in the genus *Russula*. FRIES (1838) described also *R. linnaei* based on LINNAEUS's (1753) unnamed variety *Agaricus integer* "β". RICKEN (1911) and VELENOVSKÝ (1920) considered *R. linnaei* as closely related to *R. xerampelina*. MELZER & ZVÁRA (1927a) transferred *R. linnaei* to the rank of variety into *R. xerampelina*. The name *R. erythropoda* was validly published by PELTEREAU (1908). The majority of modern authors synonymize *R. erythropoda* and *R. xerampelina* (e.g. ROMAGNESI 1967).

Many authors consider variability in section *Xerampelinae* as infraspecific and they treat all taxa of this section as varieties of *R. xerampelina* (for example MAIRE 1910). Majority of modern authors synonymize *R. erythropoda* and *R. xerampelina* (e.g. ROMAGNESI 1967).

Later other taxa were described from the range of *R. xerampelina* with purple, purple-brown or red-brown pileus cuticle and associated with coniferous trees: *R. atrosanguinea* Velen. (VELENOVSKÝ 1920), *R. fuscoochracea* Schulz (MICHAEL & SCHULZ 1926), *R. xerampelina* var. *putorina* Melzer, nom. inval. (MELZER 1945), *R. favrei* MOSER (1979). Only *R. favrei* was accepted more times by different authors (BON 1988, KNUDSEN & STORDAL 1992). REUMAUX & al. (1996) chose a very narrow species concept for this group. They accepted *R. atrosanguinea*, *R. fuscoochracea*, *R. favrei*, *R. abietum*, *R. amoenipes* and described several new related taxa (although three of them under invalid names according to International Code of Botanical Nomenclature (GREUTER & al. 2000) art. 36.1, 34.1b): *R. rivulosa* Reumaux, Bidaud & Moënne-Loccoz, nom. inval., *R. brevissima* Moënne-Loccoz, *R. suberythropus* Moënne-Loccoz, nom. inval., *R. subpurpurea* Reumaux, nom. inval.

Some authors considered also taxa indicated from deciduous forests or without indication of mycorrhizal partner in original description to be conspecific or related to *R. xerampelina*: *R. xerampelina* var. *erythropoda* f. *subrubens* (J. E. Lange) J. Blum, nom. inval. (BLUM 1962), *R. xerampelina* var. *rubra* (Britzelm.) Singer (SINGER 1926, SCHAEFFER 1933), *R. purpurea* (Schaeff.) Gillet (LANGE 1926, 1938; REUMAUX & al. 1996), *R. xerampelina* var. *fusca* (Quél.) Melzer & Zvára (KÜHNER 1953) and *R. xerampelina* var. *quercetorum* Singer (BLUM 1961, 1962).

The aim of this study is to find out whether *R. xerampelina* and *R. erythropoda* are indeed conspecific. This was done by using morphometric analyses of fruitbodies of the *R. xerampelina* group collected in Slovakia. The relation between these two species and later described taxa, as well as the nomenclature of this group will be presented in the second part of this series of papers (ADAMČÍK 2001).

## Material and methods

Sixty seven fruitbodies of *Russula* sect. *Xerampelinae* with a purple, purple-brown and red-brown pileus, and associated with coniferous trees were collected from ten localities in Slovakia for the morphometric analyses of the *R. xerampelina* group in 1998 (Tab. 1). These collections represent two distinct morphotypes: one with purple-brown or red-brown and the other one with purple colour on pileus margin, labelled as group A and group B.

Tab. 1. **List of localities** of collections of the *R. xerampelina* group with their geographical origin, date of collection and number of fruitbodies used for morphometric analyses

**Group A - collections with purple-brown or red-brown pileus cuticle.**

1. Západné Tatry mountains, at Jalovecký potok stream, in the mouth of Jalovecká dolina valley, under *Picea abies*, *Larix decidua*, on the decayed needles among the sparse herbal undergrowth, ca. 730 m a.s.l., 26.VI.1998 - 10 fruitbodies.
2. Západné Tatry mountains, in the firth of Bobrovecká dolina, under *Picea abies*, in the dense herbal undergrowth of *Melampyrum silvaticum*, *Alchemilla* sp., *Poa* sp. and another plants, ca. 750 m a.s.l., 26.VI.1998 - 4 fruitbodies.
3. Veľká Fatra mountains, Bukovinka hill, under *Picea abies*, on the decayed needles in the sparse herbal undergrowth, 780 m a.s.l., 16.VII.1998 - 11 fruitbodies.
4. Nízke Tatry mountains, on the pasture above Brankovský vodopád falls, under *Picea abies*, in decayed needles, ca. 1000 m a.s.l., 16.VII.1998 - 1 fruitbody.

**Group B - collections with purple pileus cuticle.**

1. Záhorská nížina lowland, between Tomky village and Červený rybník lake, 5 km W of Lakšárska Nová Ves village, on the sandy soil, among mosses and grass on the margin of the path, under *Pinus silvestris*, 200 m a.s.l., 30.IX.1998 - 7 fruitbodies.
2. Záhorská nížina lowland, Červený rybník lake, 3 km W of Lakšárska Nová Ves village, on the sandy soil, among mosses, under *Pinus silvestris*, 200 m a.s.l., 30.IX.1998 - 5 fruitbodies.
3. Nízke Beskydy mountains, Ondavská vrchovina hills, 2 km E of Kvakovce village, under *Pinus silvestris*, on the ground without herbal undergrowth, 200 m a.s.l., 7.X.1998 - 10 fruitbodies.
4. Nízke Tatry mountains, 1 km S of the margin the town of Liptovský Hrádok, under *Pinus silvestris* and *Picea abies*, on decayed needles, 775 m a.s.l., 14.X.1998 - 12 fruitbodies.
5. Nízke Tatry mountains, in the valley 1 km SSW the town of Liptovský Hrádok, under *Picea abies*, in decayed needles and among grass, 725 m a.s.l., 14.X.1998 - 4 fruitbodies.
6. Liptovská kotlina , 1 km S of Východná village, on the right side of river Váh, under *Picea abies*, among moses, 860 m a.s.l., 15.X.1998 - 3 fruitbodies.

The voucher specimens are deposited in herbarium SAV (each fruitbody represents a specimen). For each fruitbody 31 macromorphological and 30 micromorphological characters were measured or scored. Macromorphological characters were observed in fresh condition. Some of them varied during maturation. The mean values of macromorphological characters of juvenile and mature fruitbodies were compared in both groups. The juvenile fruitbodies have neither well developed pileus (having its margin curved down), nor spore print and mature spores, and have strong reaction of flesh to $FeSO_4$. The significant differences between juvenile and mature fruitbodies were observed on the following characters (for their definition see Tab. 2, number of characters in brackets): diameter of pileus (1), striature on the margin of pileus (2), peeling of the pileus cuticle on the margin (5), length of stipe (10), thickness of stipe (11), shape of stipe (12), striature on the surface of stipe (13) and thickness of flesh in the middle of pileus radius (26). In the case of juvenile fruitbodies, values of these characters were replaced with mean values of mature fruitbodies of the corresponding group of localities. Juvenile fruitbodies have no spore print and the colour of spore print (27) was replaced by the median value of mature fruitbodies of the corresponding group of localities, because of the majority of mature fruitbodies reached the same value.

Tab. 2. **List; of macromorphological characters.**

1. Diameter of pileus [in cm]. Majority of fruitbodies have the pileus of irregular shape and in such cases mean value of maximum and minimum width was calculated.
2. Striature on the margin of pileus [in mm from the margin to the centre pileus].
3. Tarnish of pileus cuticle [cuticle mat: 1- only on the margin of pileus, 2 - to the middle of pileus radius, 3 - almost all pileus matt].
4. Relief of surface of pileus cuticle in the centre [0 - smooth, 1 - rough and/or wrinkled, 2 - scrobiculated].
5. Peeling of pileus cuticle on the margin [in mm from the margin to the centre pileus]
6. Presence of purple colour on the margin of pileus cuticle: 7pe, 7pg, 7pi, 7pl, 7ng, or 7nc according to the colour tables UNESMA [0 - absent, 1 - present].
7. Presence of brown colour on the pileus cuticle: 3le, 3ni, 3pi, 4pg, 4pi, 5ng or 5pi according to the colour tables UNESMA [0 – absent or present only on spots, 1 - brown is one from dominant colour].
8. Contrast of coloration of pileus cuticle on the margin and in the centre [0 - cuticle is lighter on the margin of pileus, 1 - cuticle on the margin is darker or the same colour intensity as in the centre of pileus].
9. Discoloration of pileus cuticle after chaffing with the wet filter paper [the pigment trace on the paper is: 1 - rose to purple, 2 - brown-rose, 3 - brown].
10. Length of stipe [in cm].
11. Thickness of stipe [in cm]. If the stipe was not cylindrical, then average value of maximum and minimum thickness was recorded.
12. Shape of stipe [1 - narrowed on the base, 2 - cylindrical, 3 - clavate].
13. Striature on the surface of spite [1 - obscure, 2 - fine but distinct, 3 - rough or deeply striate].
14. Presence of rose colour on the stipe surface [0 - the surface is completely white, 1 - rose spot does not cover more than half of the surface, 2 - approximately half of the surface is of rose colour, 3 - the major part of surface is of rose colour].
15. Thickness of cortex of stipe [in mm].
16. Width of lamellae [in mm].
17. Number of forked lamellae near the stipe.
18. Number of forked lamellae in the central zone of hymenium.
19. Number of forked lamellae near the margin of pileus.
20. Total number of forked lamellae in the hymenium.
21. Number of lamellulae longer than half of length of lamellae.
22. Number of lamellulae approximately as long as half of length of lamellae.
23. Number of lamellulae shorter than half of length of lamellae.
24. Total number of lamellulae.
25. Total number of lamellae in hymenium. For simplification only lamellae on the 1 cm on the pileus margin were counted. The total number of lamellae is defined as the number of lamellae on 1 cm of the pileus margin multiplied by perimeter in cm.
26. Thickness of flesh in the half of radius of pileus [in mm].
27. Colour of spore print according to Romagnesi's scale (ROMAGNESI, 1967) [1 - IIIa, 2 - IIIb, 3 - IIIc, 4 - IVa].
28. Ratio of characters 1 and 5.
29. Ratio of characters 1 and 10.
30. Ratio of characters 1 and 11.
31. Ratio of characters 1 and 26.

All micromorphological characters were observed under oil-immersion lens at a magnification of 1,600×. Basidia and pleurocystidia were observed in a solution of ammonia and Congo Red (according to FÁBRY 1979), spores were observed in Melzer's reagent (MELZER & ZVÁRA 1945), hyphae of pileus epicutis were observed in a solution of sulphorvanilline (MELZER & ZVÁRA 1945).

The value of each micromorphological character was calculated as average of 10 measurements. However, it was not possible to measure 10 measurements on each fruitbody or even one for some characters (young fruitbodies had not developed mature spores, old fruitbodies had often deformed elements in hymenium layer by initial decay or the specimen was poorly dried). These failures of measuring were replaced as in the case of macromorphological characters. Absent values were replaced by the mean values of all measurements of appropriate character observed on fruitbodies from the same locality and collection. If less than 10 such measurements were made altogether on the material from one locality, absent values were replaced by mean value of all measurements of particular character for appropriate group of localities. For the ratio of length and width of basidia, pleurocystidia and spores mean value of the 10 ratios is presented. The measuring accuracy is per 0.5 µm except the size of spores and length of spines, which were measured with accuracy per 0.1 µm.

Tab. 3. **List of micromorphological characters**

32. Length of basidia [in µm].
33. Width of basidia [in µm].
34. Ratio of characters 32 and 33.
35. Length of pleurocystidia [in µm].
36. Width of pleurocystidia [in µm].
37. Ratio of characters 35 and 36.
38. Length of appendix of pleurocystidia [in µm, if the appendix is absent than the value is 0]. Appendix is narrowed terminal part of pleurocystidia, which have not granular content in sulphorvanillin and is easily broken.
39. Presence of constriction on the terminal part of pleurocystidia [0 - absent, 1 - present]. Constriction is present, if the pleurocystidium is not equally narrowed, and on some places neck is formed. Content of pleurocystidia in the place of constriction is granular.
40. Termination of pleurocystidia [0 - rounded, 1 - acute].
41. Length of spores [in µm].
42. Width of spores [in µm].
43. Ratio of characters 41 and 42.
44. Length of spines [in µm].
45. Number of spines of spore ornamentation in the circle of diameter of 3 µm.
46. Number of amyloid punctations on the surface of spores in the circle of diameter of 3 µm, which are markedly shorter that normal spines.
47. Number of fine lines connections on the surface of spores in the circle of diameter of 3 µm.
48. Number of contact connections (not lines connection) of spines or amyloid punctations in the circle of diameter of 3 µm on the surface of spores.
49. Sum of characters 47 and 48.
50. Length of terminal cells of undifferentiated hyphae on the **margin** of pileus epicutis [in µm].
51. Width of terminal cells of undifferentiated hyphae on the **margin** of pileus epicutis [in µm].
52. Presence of attenuate terminal cells of undifferentiated hyphae on the **margin** of pileus epicutis with the terminal part narrower than 3 µm [0 - absent, 1 - present].
53. Contrast between cell width in terminal and basal part of terminal cells of undifferentiated hyphae on the **margin** of pileus epicutis [subtraction of the width in terminal and basal part is: 1 - less or equal to -3; 2 - more than -3 a less or equal to -2; 3 - more than -2 and less or equal to -1; 4 - more than -1 and less than 1; 5 - more or equal to 1 and less than 2; 6 - more or equal to 2 and less than 3; 7 - more or equal to 3].
54. Length of terminal cells of pileocystidia on the **margin** of pileus epicutis [in µm].
55. Width of terminal cells of pileocystidia on the **margin** of pileus epicutis [in µm].
56. Length of terminal cells of undifferentiated hyphae on the **centre** of pileus epicutis [in µm].
57. Width of terminal cells of undifferentiated hyphae on the **centre** of pileus epicutis [in µm].
58. Presence of attenuate terminal cells of undifferentiated hyphae on the **centre** of pileus epicutis with the terminal part narrower than 3 µm [0 - absent, 1 - present].

Tab. 3 (continued)

59. Contrast between cell width in terminal and basal part of terminal cells of undifferentiated hyphae on the **centre** of pileus epicutis [subtraction of the width in terminal and basal part is: 1 - less or equal to -3; 2 - more than -3 and less or equal to -2; 3 - more than -2 and less or equal to -1; 4 - more than -1 and less than 1; 5 - more or equal to 1 and less than 2; 6 - more or equal to 2 and less than 3; 7 - more or equal to 3].
60. Length of terminal cells of pileocystidia on the **centre** of pileus epicutis [in µm].
61. Width of terminal cells of pileocystidia on the **centre** of pileus epicutis [in µm].

Many of characters traditionally used for the identification of taxa within the *R. xerampelina* group are vaguely defined. Such characters were replaced with another, more appropriate ones or their interpretation was modified (for their definitions see Tab. 2 and Tab. 3): characters no. 3, 4, 6, 7, 8, 12, 13, 14, 27, 38, 39, 40. All these characters are qualitative and the values had to be coded for the purpose of morphometric analyses.

The usually used character of colour of flesh under pileus cuticle was replaced with discoloration of pileus cuticle after chaffing with the wet filter paper (character no. 9), because both these characters indicate ability of discoloration of cuticle pigments, but the character 9 is more precise (see Tab. 2). Thickness of cortex of stipe (15) replaces the hardness of pileus (determined by many authors as "firm", "fragile", "flexible", ...). Similar relations are between hardness of pileus and thickness of flesh in the half of radius of pileus (26). Density of lamellae, forkations and lamellulae are traditionally interpreted as "often forked", "with numerous lamellulae", "sparse lamellae" and so on. The interpretation of these characters was modified: the total number of corresponding elements was scored (characters no. 20, 24, 25). However, the lamellulae have different length and the forkations have different position. Consequently, these elements were scored in different positions on the hymenium (characters 17, 18, 19, 21, 22, 23). Some features are frequently indicated as relative, such as "strong striature on the pileus margin", "short stipe", "thick stipe" or "fleshy pileus". These relative characters were treated here as ratio of two characters with numerical values (28, 29, 30, 31).

For interpretation of spore ornamentation scales had been utilized (for example MELZER & ZVÁRA 1945, CRAWSHAY 1930) or terminology reflected degree of reticulation and height of ornamentation. All characters traditionally used for descriptions of spore ornamentation are more precisely defined here. Single elements of spore ornamentation were observed in the circle of diameter of 3 µm on the spore surface in the upper plane of focus (Fig. 1.a). The ornamentation was drawn from this circle into schematic chart (Fig. 1.b), where the particular elements were numbered (characters no. 45, 46, 47, 48, 49; for their definition see Tab. 3).

The exploitation of different character of cuticle on the centre and on the margin of pileus for taxonomy of *Russula* species had been very rare (e.g. ROMAGNESI (1967) identified this feature in the section *Heterophyllae* (Fr.) Jul. Schäff., and supposed its presence in the section *Xerampelinae*). The traditionally used character of hyphae in pileus epicutis, namely multiplicity of septae was substituted with multiple measuring of the terminal cells, because the multiplicity of septae depends on quality of preparation. The shape of terminal cells of hyphae is taken into consideration by characters no. 53 and 59 (for definition of characters see Tab. 3).

The characters without distinct differences among groups were also observed in this study, such as flexibility of flesh, shape of pileus on the cut-section, intensity of reaction of flesh to $FeSO_4$, colour of stipe flesh, staining of flesh after bruising, shape of lamellae, connection of lamellae on the stipe, coloration of edge of lamellae, length of sterigmata, abundance of pleurocystidia, size of hilar appendix or size of hilar plage. Frequently some of these characters (macromorphological ones) change during maturation of fruitbody of *Russula* sect. *Xerampelinae*, but in spite of it many mycologists take them into consideration. Characters without distinct differences between groups, were not used in morphometric analyses. Visibility of intervenation of lamellae depends on density of lamellae (25; for its definition see Tab. 2) and colour of lamellae depends on colour of spore print in mature (27), hence these two characters were not used in morphometric analyses either.

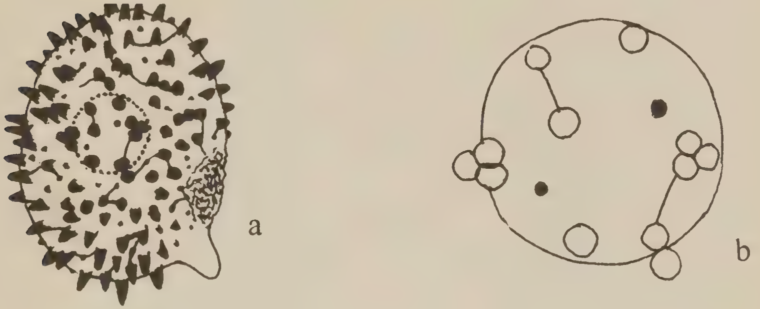

Fig. 1. a. - Schematic marking of the circle of diameter of 3 µm on the surface of spore of *Russula* species with the dotted line. b. - Schematic chart of the spore ornamentation in the circle of diameter 3 µm on the surface of spore of Russula species. O - spines; • - amyloid punctations; lines - fine line connections. In this case in the circle are: 9 spines, 2 amyloid punctations, 2 line connections and 3 contact connections.

The multivariate morphometric analyses were performed in the following steps:
1. In order to generate a hypothesis suggesting possible grouping of observed fruitbodies, principal components analysis (PCA) (SNEATH & SOKAL 1973, KRZANOWSKI 1990) and principal co-ordinates analysis (PCoA were used in the case that number of variables exceed number of fruitbodies) were performed.
2. Correlation coefficient (Pearson coefficient) express relations between characters.
3. Canonical discriminant analyses (CDA) (KLECKA 1980, KRZANOWSKI 1990) were used for verification of the obtained hypotheses.
4. Exploratory data analyses (EDA) (TUKEY 1977, SAS INSTITUTE 1990a) were carried out on the data matrices of groups resulting as a hypothesis from the PCA.

All analyses except PCoA were performed using procedures PRINCOMP, UNIVARIATE, CORR and CANDISC, available in the SAS package (SAS INSTITUTE 1990a, b) at the Department of Botany of the Charles University, Prague. PCoA were performed by program PRINCOOR using the SYNTAX package (PODANI 1993) at the Institute of Botany, Slovak Academy of Sciences, Bratislava.

### Results of morphometric analyses

Results of principal components analysis (PCA) on the three dimensional ordination diagram (Fig. 2) present apparent difference between two groups of fruitbodies, separated along the first component, which are possible to identify with group A and B (listed in Tab. 1). The first axis accounts for 31.9%, the second for 8.6% and the third for 7% of the variance. Among the most strongly correlated characters with the first component are especially micromorphological ones (for their definition see Tab. 3) describing shape and width of terminal cells of undifferentiated hyphae on the pileus epicutis (characters no. 51, 52, 53, 57, 58), size and spore ornamentation (42, 43, 45, 47, 48, 49), shape of terminal part of pleurocystidia (38, 39, 40) and width of pileocystidia (55, 61). The first component is less affected by macromorphological characters, only the characters 6, 7, 8, 9, 11, 21 and 27 have stronger influence on this axis (for their definition see Tab. 2). Fruitbodies of group B were collected on sandy soil in Záhorská nížina lowland, in submountain zone of the Nízke Tatry mountains and in colline zone of the Ondavská vrchovina hills. Fruitbodies from Záhorská nížina lowland are separated from the other fruitbodies of this group along the third component. With this component mainly macromorphological characters (15, 20, 25, 26, 29, 31, 44) are strongly correlated, from micromorphological characters only length of spores have strong influence on this component. Fruitbodies of group A does not form any ecologically or geographically defined groups on the PCA diagram.

Pearson correlation coefficients among characters in the data matrices of individual groups A and B of are not either very highly (exceeding value 0.95) or highly correlated (exceeding value 0.9). Values of Pearson correlation coefficient are highly correlated only between characters 6-52 and 41-42 in the pooled data matrix of both groups, thus all above-mentioned characters can be used in canonical discriminant analysis.

The results of canonical discriminant analysis (CDA) of group A and group B are similar to those of the PCA. The studied groups are apparently separated along the canonical axis (Fig. 3). The total canonical structure express correlation of individual characters with canonical axis and the strongly correlated characters are important for separation of these two groups (and obviously can be used as discriminant characters). All characters with high value of total canonical structure have strong influence to separation of these groups by PCA as well (Tab. 4).

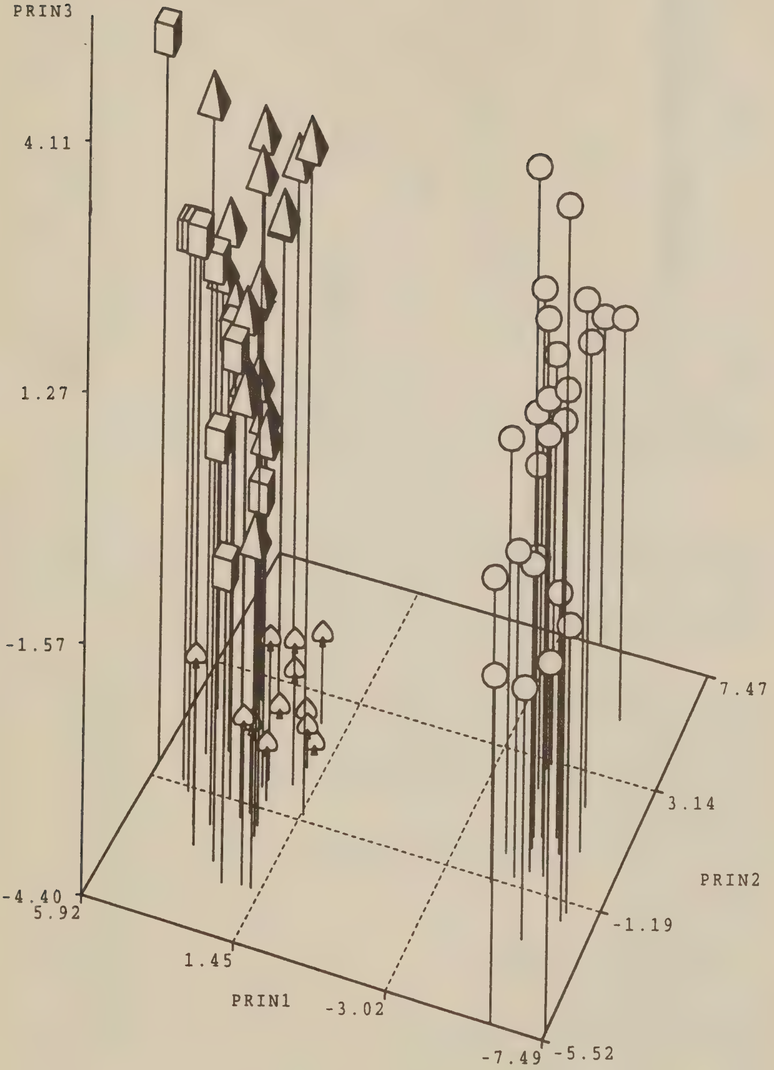

Fig. 2. Ordination diagram of principal component analysis of the fruitbodies of the *Russula xerampelina* group with purple-brown or red-brown pileus cuticle (group A) and purple pileus cuticle (group B). Ball – group A; pyramid - group B from the Nízke Tatry mountains; cube – group B from the Ondavská vrchovina hills; spade – group B from Záhorská nížina lowland.

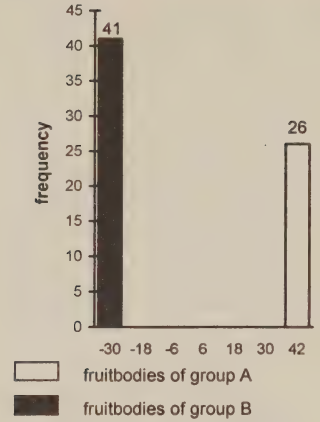

Fig. 3. Ordination diagram of the canonical discriminant analysis of the fruitbodies of group A and B (the groups are listed in Tab. 1).

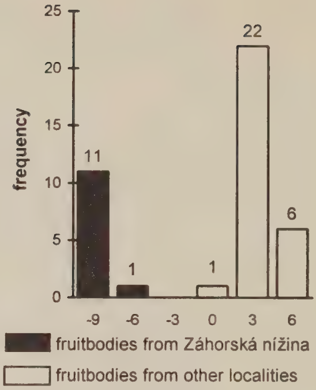

Fig. 4. Ordination diagram of the canonical discriminant analysis of the fruitbodies of group B (listed in Tab. 1) from Záhorská nížina lowland and other localities.

Tab. 4. Total canonical structure exceeding value of 0.6 obtained in the canonical discriminant analysis of the fruitbodies of the *Russula xerampelina* group with purple-brown or red-brown pileus cuticle (group A) and purple pileus cuticle (group B). CIR - the circle of diameter of 3 µm on the surface of spores. TC - terminal cells of undifferentiated hyphae in pileus epicutis. For detail descriptions of characters see Tab. 2 and 3.

| **Characters** | **Total canonical structure** |
|---|---|
| 6. Presence of purple colour on the margin of pileus cuticle | -0.940189 |
| 7. Presence of brown colour on the pileus cuticle | 0.703635 |
| 8. Contrast of coloration of pileus cuticle on the margin and in the centre | 0.645652 |
| 9. Discoloration of pileus cuticle after chaffing with the wet filter paper | 0.833022 |
| 21. Number of lamellulae longer than half of length of lamellae | -0.634944 |
| 27. Colour of spore print | -0.865082 |
| 38. Length of appendix of pleurocystidia | -0.694245 |
| 39. Presence of constriction on the terminal part of pleurocystidia | -0.767592 |
| 40. Termination of pleurocystidia | -0.8976 |
| 41. Length of spores | 0.681156 |
| 42. Width of spores | 0.774062 |
| 45. Number of spines in CIR | -0.722996 |
| 47. Number of fine lines connections in CIR | -0.723094 |
| 48. Number of contact connections in CIR | -0.735017 |
| 49. Sum of characters 47 and 48 | -0.837262 |
| 51. Width of TC on the margin | 0.879982 |
| 52. Presence of attenuate TC on the margin | -0.986016 |
| 53. Contrast between cell width in terminal and basal part of TC on the margin | 0.887761 |
| 55. Width of terminal cells of pileocystidia on the margin of pileus epicutis | 0.847824 |
| 57. Width of TC on the centre | 0.778253 |
| 58. Presence of attenuate TC on the centre | -0.830184 |
| 61. Width of terminal cells of pileocystidia on the centre of pileus epicutis | 0.883081 |

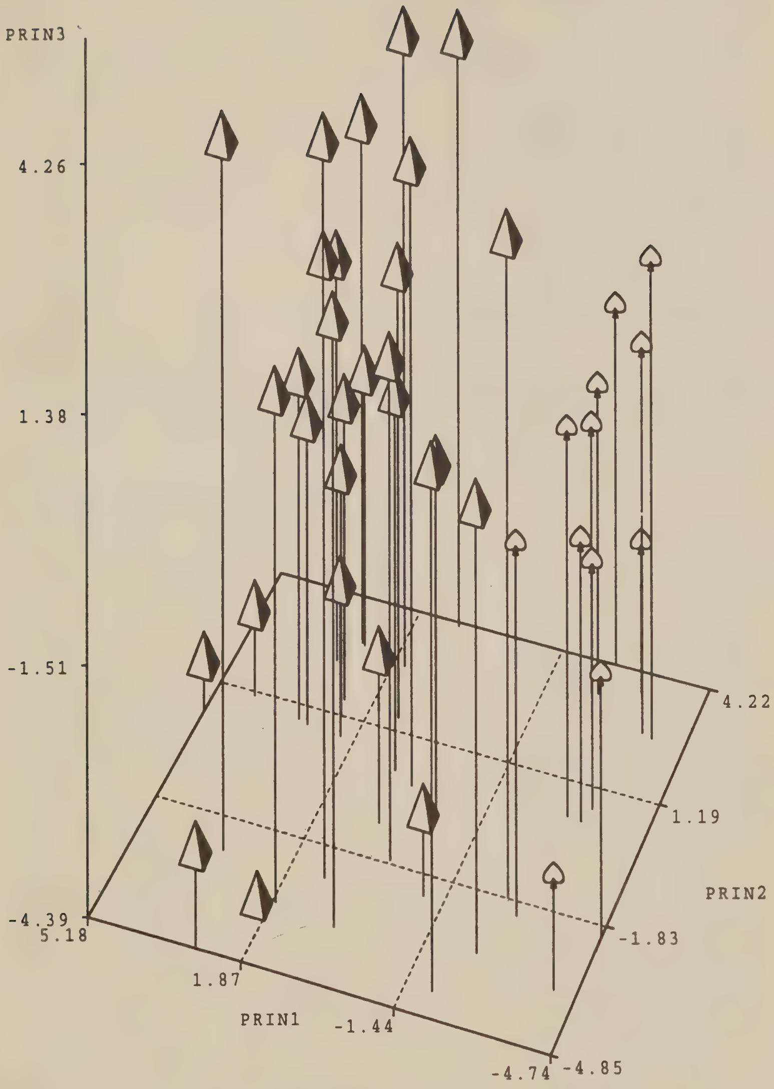

Fig. 5. Ordination diagram of principal co-ordinates analysis of fruitbodies of the *Russula xerampelina* group with purple pileus cuticle (group B) from Záhorská nížina lowland (spade) and from other localities (pyramid).

Tab. 5. Total canonical structure exceeding value of 0.4 obtained in the canonical discriminant analysis of fruitbodies of the *Russula xerampelina* group with purple pileus cuticle (group B) from Záhorská nížina lowland and other localities. CIR - the circle of diameter of 3 µm on the surface of spores. TC - terminal cells of undifferentiated hyphae in pileus epicutis. For detail descriptions of characters see Tab. 2 and 3.

| **Characters** | **Total canonical structure** |
|---|---|
| 2. Striature on the margin of pileus | -0.534046 |
| 15. Thickness of cortex of stipe | 0.49823 |
| 21. Number of lamellulae longer than half of length of lamellae | 0.430309 |
| 25. Total number of lamellae | 0.55224 |
| 26. Thickness of flesh in the half of radius of pileus | 0.732159 |
| 32. Length of basidia | 0.477031 |
| 34. Ratio of characters 32 and 33 | 0.429844 |
| 41. Length of spores | 0.404417 |
| 43. Ratio of characters 41 and 42 | 0.456598 |
| 56. Length of TC on the centre | 0.565987 |
| 59. Contrast between cell width in terminal and basal part of TC on the centre | -0.432511 |

Tab 6. Results of exploratory data analysis for selected characters with distinct difference between the fruitbodies of the *Russula xerampelina* group with purple-brown or red-brown pileus cuticle (group A - 26 fruitbodies) and purple pileus cuticle (group B - 41 fruitbodies). CIR - the circle of diameter 3 µm on the surface of spores. TC - terminal cells of undifferentiated hyphae in pileus epicutis. For detail descriptions of characters see Tab. 2 and Tab. 3.

| **Characters** | **Group** | **Mean** | **Median** | **Standard deviation** | **Percentiles** | |
|---|---|---|---|---|---|---|
| | | | | | **95%** | **5%** |
| 1. Diameter of pileus | A | 5.6 | 5.6 | 1.51 | 8 | 2.6 |
| | B | 7.23 | 7.23 | 1.49 | 9.5 | 4.8 |
| 3. Tarnish of pileus cuticle | A | 1.69 | 2 | 0.68 | 3 | 1 |
| | B | 1.1 | 1 | 0.44 | 2 | 1 |
| 4. Relief of surface of pileus cuticle in the centre | A | 1.58 | 2 | 0.5 | 2 | 1 |
| | B | 1.12 | 1 | 0.46 | 2 | 1 |
| 6. Presence of purple colour on the margin of pileus cuticle | A | 0 | 0 | 0 | 0 | 0 |
| | B | 0.95 | 1 | 0.22 | 1 | 1 |
| 7. Presence of brown colour on the pileus cuticle | A | 0.62 | 1 | 0.5 | 1 | 0 |
| | B | 0 | 0 | 0 | 0 | 0 |
| 8. Contrast of coloration of pileus cuticle on the margin and in the centre | A | 0.54 | 1 | 0.51 | 1 | 0 |
| | B | 0 | 0 | 0 | 0 | 0 |
| 9. Discoloration of pileus cuticle after chaffing with the wet filter paper | A | 2.42 | 2 | 0.5 | 3 | 2 |
| | B | 1.15 | 1 | 0.36 | 2 | 1 |
| 10. Length of stipe | A | 4.28 | 4.28 | 1.01 | 6.5 | 2.4 |
| | B | 5.71 | 5.4 | 1.56 | 8.7 | 4 |
| 11. Thickness of stipe | A | 1.4 | 1.4 | 0.38 | 2.3 | 0.9 |
| | B | 2 | 1.9 | 0.42 | 2.8 | 1.4 |
| 14. Presence of rose colour on the stipe surface | A | 1.46 | 0 | 1.17 | 3 | 0 |
| | B | 2.32 | 3 | 0.91 | 3 | 1 |
| 21. Number of lamellulae longer than half of length of lamellae | A | 0.15 | 0 | 0.54 | 2 | 0 |
| | B | 3.12 | 3 | 2.23 | 7 | 0 |
| 26. Thickness of flesh in the half of radius of pileus | A | 3.09 | 3.09 | 0.67 | 4.5 | 2 |
| | B | 4.3 | 4.5 | 1.4 | 6.5 | 2 |
| 27. Colour of spore print | A | 2 | 2 | 0.28 | 2 | 2 |
| | B | 3.05 | 3 | 0.31 | 4 | 3 |
| 28. Ratio of characters 1 and 5 | A | 0.37 | 0.34 | 0.13 | 0.61 | 0.2 |
| | B | 0.56 | 0.58 | 0.17 | 0.84 | 0.35 |

Tab. 6 (continued)

| Characters | Group | Mean | Median | Standard deviation | Percentiles | |
|---|---|---|---|---|---|---|
| | | | | | 95% | 5% |
| 32. Length of basidia | A | 49.79 | 36.95 | 6.2 | 58.75 | 38.35 |
| | B | 45.31 | 43.95 | 5.73 | 56.55 | 37.4 |
| 33. Width of basidia | A | 13.42 | 13.45 | 1.12 | 15.05 | 11.45 |
| | B | 12.32 | 12.25 | 0.62 | 13.4 | 11.25 |
| 38. Length of appendix of pleurocystidia | A | 0.33 | 0 | 0.71 | 2 | 0 |
| | B | 5.51 | 5.47 | 3.34 | 10.05 | 1.45 |
| 39. Presence of constriction on the terminal part of pleurocystidia | A | 0.21 | 0.13 | 0.19 | 0.5 | 0 |
| | B | 0.66 | 0.65 | 0.19 | 1 | 0.4 |
| 40. Termination of pleurocystidia | A | 0.33 | 0.3 | 0.24 | 0.8 | 0 |
| | B | 0.96 | 0.98 | 0.06 | 1 | 0.9 |
| 41. Length of spores | A | 9.57 | 9.52 | 0.44 | 10.27 | 9.01 |
| | B | 8.81 | 8.87 | 0.38 | 9.24 | 8.25 |
| 42. Width of spores | A | 7.63 | 7.64 | 0.29 | 8.18 | 7.22 |
| | B | 6.93 | 6.93 | 0.28 | 7.35 | 6.52 |
| 43. Ratio of characters 41 and 42 | A | 1.25 | 1.25 | 0.03 | 1.31 | 1.22 |
| | B | 1.27 | 1.28 | 0.02 | 1.3 | 1.23 |
| 45. Number of spines in CIR | A | 5.78 | 5.68 | 0.86 | 7.05 | 4.45 |
| | B | 7.53 | 7.65 | 0.81 | 8.7 | 5.95 |
| 47. Number of fine lines connections in CIR | A | 1.14 | 1.04 | 0.61 | 2.4 | 0.4 |
| | B | 2.81 | 2.8 | 0.88 | 4.4 | 1.55 |
| 48. Number of contact connections in CIR | A | 3.54 | 3.43 | 0.96 | 4.85 | 2 |
| | B | 5.6 | 5.75 | 0.93 | 6.8 | 4.15 |
| 49. Sum of characters 47 and 48 | A | 4.68 | 4.47 | 1.4 | 6.2 | 2.5 |
| | B | 8.41 | 8.5 | 1.07 | 9.75 | 6.95 |
| 50. Length of TC on the margin | A | 25.8 | 25.03 | 3.43 | 31.75 | 20.35 |
| | B | 32.17 | 31.25 | 4.89 | 39.7 | 26.05 |
| 51. Width of TC on the margin | A | 5.84 | 5.91 | 0.53 | 6.5 | 5.05 |
| | B | 4.2 | 4.25 | 0.37 | 4.7 | 3.6 |
| 52. Presence of attenuate TC on the margin | A | 0.04 | 0.01 | 0.05 | 0.1 | 0 |
| | B | 0.96 | 1 | 0.09 | 1 | 0.7 |
| 53. Contrast between cell width in terminal and basal part of TC on the margin | A | 4.23 | 4.19 | 0.51 | 5.1 | 3.72 |
| | B | 2.31 | 2.3 | 0.48 | 3 | 1.5 |
| 55. Width of terminal cells of pileocystidia on the margin of pileus epicutis | A | 7.58 | 7.58 | 0.71 | 8.67 | 6.33 |
| | B | 5.48 | 5.42 | 0.61 | 6.67 | 4.67 |
| 56. Length of TC on the centre | A | 20.62 | 20.94 | 2.55 | 24.7 | 16.85 |
| | B | 24 | 24.15 | 3.17 | 30 | 19.5 |
| 57. Width of TC on the centre | A | 5.86 | 5.85 | 0.67 | 7 | 4.95 |
| | B | 4.37 | 4.43 | 0.55 | 5.32 | 3.5 |
| 58. Presence of attenuate TC on the centre | A | 0.03 | 0 | 0.1 | 0.29 | 0 |
| | B | 0.6 | 0.6 | 0.23 | 0.9 | 0.23 |
| 61. Width of terminal cells of pileocystidia on the centre of pileus epicutis | A | 7.29 | 7.33 | 0.8 | 8 | 6.17 |
| | B | 4.97 | 4.93 | 0.45 | 5.67 | 4.17 |

Tab 7. Results of exploratory data analysis for selected characters with distinct difference between fruitbodies of the *Russula xerampelina* group with purple pileus cuticle (group A) from Záhorská nížina lowland (zah - 12 fruitbodies) and from other localities (oth - 29 fruitbodies). TC - terminal cells of undifferentiated hyphae in pileus epicutis. For detail descriptions of characters see Tab. 2 and 3.

| Characters | Group | Mean | Median | Standard deviation | Percentiles | |
|---|---|---|---|---|---|---|
| | | | | | 90% | 10% |
| 1. Diameter of pileus | zah | 6.67 | 6.95 | 0.9 | 7.3 | 5.7 |
| | oth | 7.47 | 7.4 | 1.63 | 9.5 | 5.3 |
| 2. Striature on the margin of pileus | zah | 7.08 | 6.5 | 2.75 | 11 | 4 |
| | oth | 4.1 | 4.97 | 2.03 | 7 | 1.5 |
| 11. Thickness of stipe | zah | 1.75 | 1.8 | 0.27 | 1.9 | 1.4 |
| | oth | 2.1 | 2 | 0.43 | 2.8 | 1.5 |
| 15. Thickness of cortex of stipe | zah | 1.58 | 1.5 | 0.29 | 2 | 1.5 |
| | oth | 2.34 | 2.12 | 0.72 | 3.5 | 1.5 |
| 16. Width of lamellae | zah | 9.29 | 10 | 2.38 | 12 | 5.5 |
| | oth | 8.28 | 8 | 2.44 | 12 | 4.5 |
| 21. Number of lamellulae longer than half of length of lamellae | zah | 1.67 | 1 | 2.1 | 5 | 0 |
| | oth | 3.72 | 4 | 2.03 | 7 | 1 |
| 25. Total number of lamellae | zah | 134.17 | 162 | 27.85 | 161 | 99 |
| | oth | 168.31 | 234 | 23.21 | 202 | 141 |
| 26. Thickness of flesh in the half of radius of pileus | zah | 2.75 | 2.75 | 0.58 | 3.5 | 2 |
| | oth | 4.94 | 4.5 | 1.1 | 6.5 | 3.5 |
| 29. Ratio of characters 1 and 10 | zah | 1.09 | 1.06 | 0.22 | 1.41 | 0.82 |
| | oth | 1.41 | 1.37 | 0.29 | 1.86 | 0.98 |
| 31. Ratio of characters 1 and 26 | zah | 2.53 | 2.38 | 0.63 | 3.28 | 1.9 |
| | oth | 1.53 | 1.53 | 0.24 | 1.87 | 1.17 |
| 32. Length of basidia | zah | 41.18 | 41.28 | 3.72 | 45.8 | 36.8 |
| | oth | 47.02 | 45.8 | 5.58 | 56.55 | 41.7 |
| 34. Ratio of characters 32 and 33 | zah | 3.4 | 3.31 | 0.33 | 3.99 | 3.09 |
| | oth | 3.83 | 6.62 | 0.47 | 4.61 | 3.34 |
| 41. Length of spores | zah | 8.58 | 8.76 | 0.53 | 8.96 | 7.61 |
| | oth | 8.91 | 8.87 | 0.25 | 9.24 | 8.6 |
| 43. Ratio of characters 41 and 42 | zah | 1.26 | 1.27 | 0.02 | 1.28 | 1.23 |
| | oth | 1.28 | 1.28 | 0.02 | 1.3 | 1.26 |
| 56. Length of TC on the centre | zah | 21.29 | 21.15 | 2.62 | 23.25 | 18.6 |
| | oth | 25.12 | 25.15 | 2.68 | 30 | 21.15 |
| 59. Contrast between cell width in terminal and basal part of TC on the center | zah | 3.44 | 3.45 | 0.3 | 3.7 | 2.9 |
| | oth | 3.07 | 3 | 0.39 | 3.6 | 2.5 |

The principal co-ordinates analysis (PCoA) of fruitbodies of group B based on Euclidean distance was performed in order to reveal in detail the differences between fruitbodies from Záhorská nížina lowland and from other localities from Slovakia (PCoA was used in this case because the number of variables exceed the number of fruitbodies) already shown on the PCA ordination diagram (Fig. 2). The PCoA confirmed this difference and on the ordination diagram (Fig. 5) these two groups are separated along the first component. The first axis accounts for 12.9%, the second 11.4% and the third 8.8% of the variance.

The number of observed characters was reduced from 61 to 39 in the canonical discriminant analysis (CDA) of fruitbodies of group B from Záhorská nížina lowland and from other localities (because the number of variables must be x-2, where x is the number of objects). The characters important for separation of group A and B in PCA and not contributing seriously to the differentiation within group B along the third component were excluded: characters no. 3, 6, 7, 8, 9, 27, 33, 38, 39, 40, 42, 45, 47, 48, 49, 51, 52, 53, 55, 57, 58, 61 (for their definition see Tab. 2 and Tab. 3). Two groups are also apparent on the ordination diagram of CDA of group B from Záhorská nížina lowland and from other localities (Fig. 4). For separation of these two groups are most important characters 2, 25, 26 and 56 (Tab. 5).

A few statistics obtained in the exploratory data analysis for selected characters with distinct difference between assigned groups are presented in Tab. 6 and Tab. 7. All characters strongly correlated with first axis of PCA of group A and B have distinct differences between these groups. Using only one character, presence of attenuate terminal cells of undifferentiated hyphae on the margin of pileus epicutis (character no. 52), it is possible correctly distinguish groups A and B. The following characters overlap in their extreme values only: 6, 9, 27, 38, 40, 42, 49, 51, 52, 55, 58 and 61. There is no such reliable character for the differentiation of fruitbodies of group B from Záhorská nížina lowland and from other localities, although it is possible to distinguish them by the combination of several characters. Some characters, which are not listed in Tab. 4 and 5, have similar minimum and maximum values for both groups, but the median and the mean values are different, such as length of basidia (32). Also characters which are stable for one group and vary for another can be useful for determination, such as presence of rose colour on the stipe surface (14).

## Discussion

Observed material, composed from 67 fruitbodies of *Russula* sect. *Xerampelinae* from selected localities in Slovakia, belongs to three groups according to the results of multivariate analyses (the group of fruitbodies with purple-brown or red-brown cuticle of pileus is labelled as "A" and the other two groups with purple cuticle of pileus as "B). There are at least 20 reliable characters suitable for determination of groups A and B. The fruitbodies can be determined using only macromorphological characters in fresh condition. Pileus cuticle of group A is often darker on the margin than in the centre and one of dominant colours is brown. On the contrary, fruitbodies of group B have only rarely darker colour on margin of pileus cuticle, the colour is pure purple on the margin and black-purple to black in the centre and brown colour is be present only in small spots. Fruitbodies of group A have brownish-rose to brownish pigment spot after chaffing of pileus cuticle with wet filter paper, whereas fruitbodies of group B have pure rose to purple colour of the pigment spot. These groups differ also in colour of spore print, which is darker in group B (bright yellow). The average value of number of lamellulae longer than half of length of lamellae is higher in group B, but this character is not suitable for discrimination of the two groups, because the average value of fruitbodies of group B from Záhorská nížina lowland is lower than in group A. The microscopic structure of almost all parts of fruitbody is different. The pleurocystidia of group B have constriction and apendix in its terminal part, and they are acute. Fruitbodies of group A have mainly rounded pleurocystidia without constriction or apendix and have larger spores with more connected and more sparse spines. Very different is structure of pileus epicutis on the margin. Fruitbodies of group A have wider clavate or cylindrical terminal cells of undifferentiated hyphae than in group B, which have attenuated terminal cells of the hyphae. Fruitbodies of group A have wider terminal cells of pileocystidia on the margin and in the centre of pileus epicutis. Both groups differ also in habit and phenology. Fruitbodies of group A occur in submontane to subalpine belt especially under *Picea* in summer (mainly from June to August). Fruitbodies of group B occur in plain to mountain coniferous forests in autumn (mainly in September to November). According to many distinct differences between groups A and B, which makes them easily recognizable, we propose to classify these groups as species.

The fruitbodies of group B from Záhorská nížina lowland form group, which is different from fruitbodies of this group from other localities in Slovakia. The macromorphological characters are contributing more to the separation of groups of fruitbodies within group B, than between both groups contrary to micromorphological ones. The fruitbodies from Záhorská nížina lowland are less fleshy: they have thiner flesh in the half of radius of pileus, thiner cortex of stipe and more striated pileus on the margin. They have widely spaced lamellae and less abundant lamellulae, which are longer than half of length of lamellae. Micromorphological differences are not so apparent like between both groups. The fruitbodies from Záhorská nížina lowland have shorter basidia, spores and terminal cells of undifferentiated hyphae on the centre of pileus epicutis. The undifferentiated hyphae are not so attenuated as in fruitbodies of group B from other localities. It is possible to determine these two groups using only micromorphological characters, but it is necessary to increase the number of measurements for confirmation of this determination. The difference between groups of fruitbodies within group B is significantly smaller than between groups A and B, and there are not such reliable characters for separation of groups within group B. Therefore, we regard variation of fruitbodies of group B as infraspecific, and we proposed fruitbodies of this group collected in pine forest in Záhorská nížina lowland on sandy soil to classify on the level of variety.

Full taxonomic account of the taxa accepted in our study is included in the second part of this series of papers ADAMČÍK (2001).

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# THE GENUS SCUTELLINIA (PEZIZALES) IN CROATIA III. A NEW SPECIES - SCUTELLINIA TUBERCULATA

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## Abstract

A new species *Scutellinia tuberculata* N. Matočec is described, which settles the warm temperate deciduous broadleaved forests in Croatia. The species is compared with morphologically most similar species and its ecology and taxonomy are discussed. *S. tuberculata* is introduced as a new member of subg. *Scutellinia*, sect. *Globisporae* Svrček.

**Key Words:** Pezizales, *Scutellinia tuberculata*, new species, taxonomy, Croatia.

## Introduction

This is the first contribution dealing with some previously unidentified material of the genus *Scutellinia* collected in Croatia, mentioned in the first paper on Croatian *Scutellinia* species (Matočec et al., 1995). One previous collection and one additional collected in 1998 were proved to be conspecific according to important characters on specific level in the genus (see also Schumacher 1990). Both collections share unique combination of characters and therefore could not match any of so far accepted species.

## Materials and Methods

Fieldwork techniques, microscopical preparation and procedures were elaborated earlier (Erb & Matheis 1983, Baral 1992, Matočec et al., 1995, Matočec 1998) and will not be repeated here. The living apothecia were microscopically examined in tap water. The ascospores obtained from fresh spore prints were firstly measured in tap water and subsequently stained in Cotton blue, in order to examine spore ornamentation. The number of measurements of the ascospores was 50 per collection, 25 for both marginal and excipular hairs and 20 for both paraphyses and asci. All characters were examined in both collections. In the species concept, organism delimitation, fructification definition, and anatomical terminology I was following our first paper (Matočec et al., 1995). Ecological observations was made relying on Bertović & Lovrić (1992).

**Abbreviations**

NM = private fungarium N. Matočec, Gajnice 13, 10000 Zagreb, Croatia, ZA = Herbarium of Department of Botany, Faculty of science, University of Zagreb, Marulićev trg 20/2; CB = cotton blue, CB+ = cyanophillic structures; CB- = non-cyanophillic structures; glob. = globose; tub. = tuberculate; (*) = living state of cell; (✝) = dead state.

***Scutellinia tuberculata*** N. Matočec, sp. nov.

*Apothecia exigua,* 1.0-1.6 *mm. lata, sessilia, cupulata, hymenio explanato, roseo vel aurantiaco; margine extus setis erectis brunneis vestita. Apothecio pili superiores* 275-795 *µm longi,* 25.0-46.5 *µm lati, acuti,* 3-11 *septis divisi, tunica ferruginea,* 2.8-5.5 *µm crassa, basi bi- vel multi-furcati. Pili inferiores* 92-285 *µm longi,* 13.5-26.0 *µm lati, acuti,* 1-5 *septis divisi, tunica lutea vel luteo-ochracea,* 0.8-3.4 *µm crassa, basi simplices vel bifurcati. Excipulum externum* 85-100 *µm crassum, e cellulis* 22.5-69.5 *µm magnis, globularibus vel angulatis compositum. Excipulum medullare* 105-125 *µm crassum, e cellulis minoribus diametro* 5.2-15.0 *µm compositum. Subhymenium indistinctum. Asci in statu vivo* 245-305 × 24.6-37.0 *µm, octospori, operculati, apice obtuso vel obtuso-truncato. Paraphysae in statu vivo basi* 2.4-3.6 *µm crassae, rectae, apice clavato-dilatatae, diametro* 6.9-16.6 *µm. Ascosporae in statu vivo diametro* 18.1-23.4 (23.8) *µm, perfecte globosae, guttulis magnis oleiferis instructae. Tunica verrucosa, verrucae magnae, tuberculatae, in statu emortuo* 1.4-5.8 (7.0) *µm latae,* 1.4-4.3 *µm altae.*
***Habitat:*** *ad terram nudam humidam in sylva decidua*
***Holotypus:*** *die 8 Mai annoi 1994 prope rivulum Okićnica in Croatia centrali, sub numero NM/2235 ab auctore lectus, Universitatis studiorum Zagrabiensis – ZA depositus; isotypi in Fungario Neven Matočec – NM depositi.*

***Scutellinia tuberculata*** N. Matočec, sp. nov.

Illustrations: Figs. 1-4.

**Apothecia** small, sessile, cupulate to shallowly cupulate but with plane hymenial surface, sessile, 1.0-1.6 mm in diam., perfectly circular from the top view. Hymenium bright orange to pinkish, never red. Apothecial margin beset with relatively obvious brownish-black hairs of medium length. Excipular surface concolorous with the hymenium or yellowish-ochre beset with much shorter hairs.

**Marginal hairs** (*) partially to totally flexuous, pointed at the apex, broadest at the basal cell, reddish-brown, uniformly coloured or basaly lighter (yellow to yellowish-ochre), 4-12 celled, 275-795 × 25.0-46.5 µm, with bi- to trifurcate base, always mixed with few longest hairs with multifurcate bases (4-5 branches of first and second order); walls 2.8-5.5 µm thick. **Hyphoid hairs** absent at the margin as well as on the excipular flanks. **Excipular hairs** (*) much shorter, flexuous, pointed to attenuated at the apex, spindle-shaped, yellow to yellowish-ochre, 1-5 celled, 92-285 × 13.5-26.0 µm with simple, unbranched to bifurcate base, walls 0.8-3.4 µm thick. **Marginal tissue** composed of subhyaline ± isodiametric angular cells, 11.6-35.4 µm in diam. **Ectal excipulum** (*) 85-100 µm thick, pseudoparenchymatic, composed of ± isodiametric globose to angular cells, 22.5-69.5 µm in diam., (✝) walls CB+. **Medullary excipulum** (*) 105-125 µm thick, composed of broad and short angular cells, 5.2-15.0 µm in diam., (✝) walls CB-. **Subhymenium** (*) not clearly differentiated from medulary excipulum. **Asci** (*) cylindric, pleurorhynchous, uniseriately filled with spores, 8-spored, operculate, obtuse to obtuse-truncate at the top, hyaline, 245-305 × 24.6-37.0 µm. **Paraphyses** (*) straight, filled with pale pinkish-orange granuliform to vermiform pigment sometimes totally absent in apical cell, normally septate, cylindric 2.4-3.8 µm in diam. at the middle, simple or branched, apically clavate, swollen apical cell 21.5-75.0 µm long and 6.9-16.6 µm in diam. **Mature ascospores** (*) perfectly globose, hyaline, 18.1-23.4 (23.8) µm, filled with smaller number of large globose lipid bodies 4.5-8.6 µm in diam., not clearly visible due to massive ornamentation. **Ascospore ornamentation** (✝) CB+ with homogenous dark blue reaction, solid, composed of very large hemisphere shaped isolated tubercles 1.4-5.8 µm in diam. and 1.4-4.3 µm in height. The outer wall not loosening when heated in CB.

**Ecology:** terricolous, on bare forest soil in mediterranoid and submediterranean forests. Eleven apothecia on steep slope above the forest path occurred in *Quercus dalechampii*, *Cornus mas*, *Carpinus betulus* and *Fraxinus ornus* forest (NM/2235), and single apothecium in humid trench in *Quercus robur*, *Cornus mas*, *Carpinus betulus* and *Acer campestre* forest (NM/4129). Associated fungi: *Helvella acetabulum* (L.: St.-Amans) Quél., *Scutellinia trechispora* (Berk. & Broome) Lambotte (with collection no. NM/2235); *Helvella ephippium* Lév. (with collection no. NM/4129).

**Etymology:** *tuberculata* (lat.) refers to specific hemisphere shaped ascospore ornamentation type.

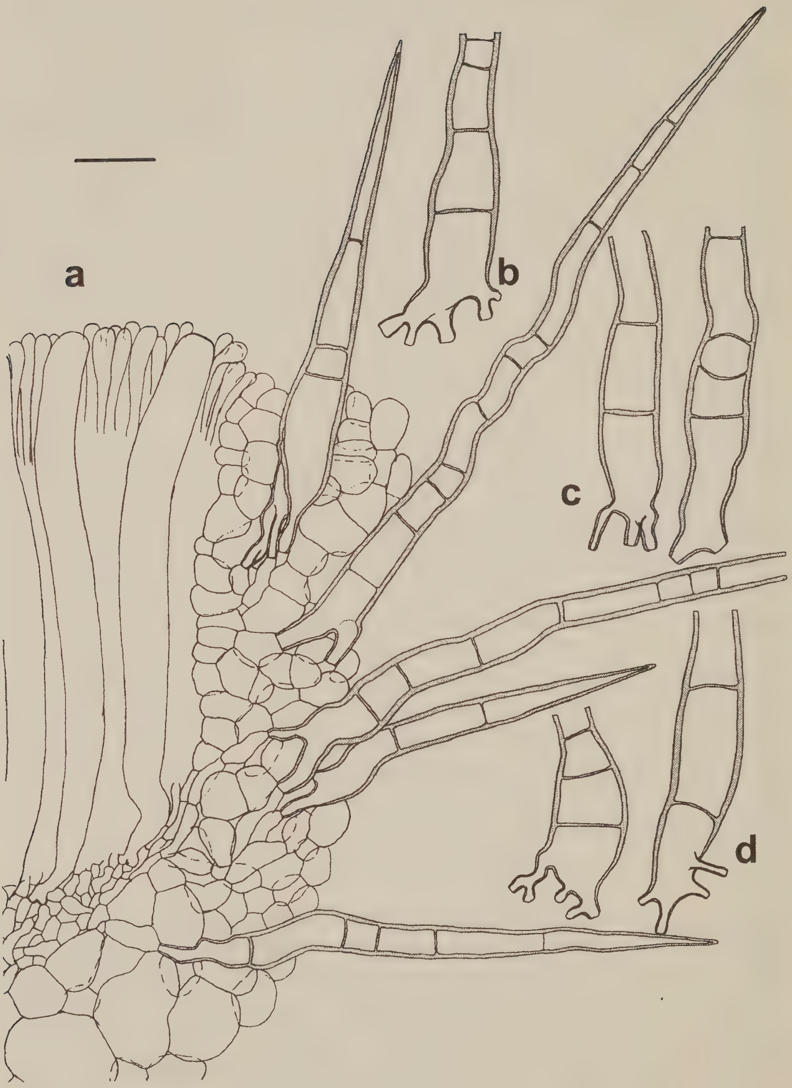

**Fig. 1.** ***Scutellinia tuberculata*** – marginal part of an apothecium with its marginal hairs (NM/4129), observed in tap water at *statu vivo*: **a** – vertical median section of the apothecium; **b-d** – marginal hair bases (bar=40µm).

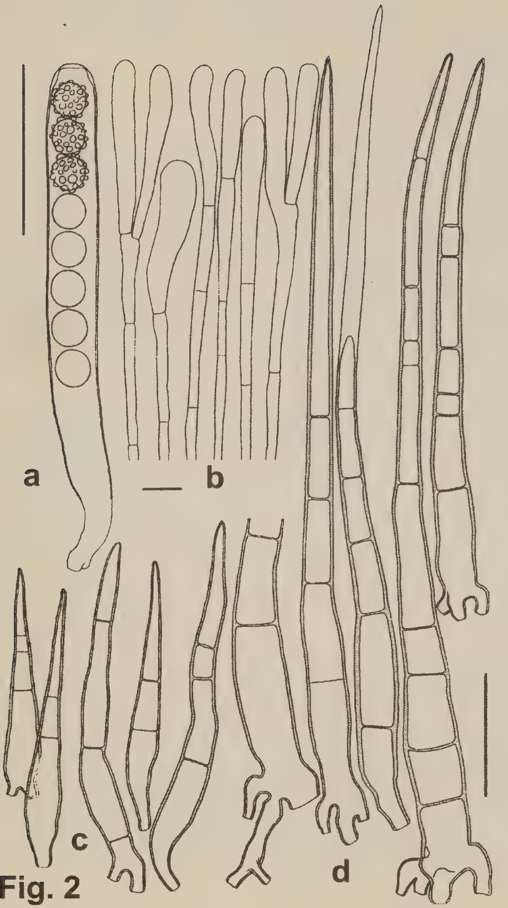

Fig. 2

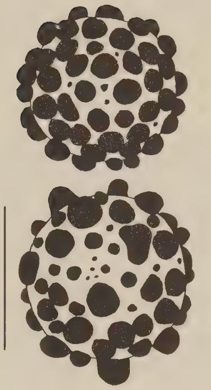

Fig. 3

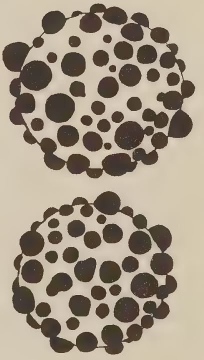

Fig.4

**Figs. 2-4**. ***Scutellinia tuberculata*** – important taxonomic characters. **Fig. 2** – hymenial elements and apothecial hairs (NM/2235, type) obtained from thin hand-section without squashing and observed in tap water at *statu vivo*: **a** – mature ascus (bar=100μm); **b** – paraphyses (bar=20μm); **c** – excipular hairs and **d** – marginal hairs (bar=100μm). **Figs. 3, 4** – ascospores from the fresh spore-print, stained in CB, observed under oil-immersion (bar=20μm). **Fig. 3** – collection no. NM/2235, type. **Fig. 4** – collection no. NM/4129.

**Collections examined:**
NM/2235 Okićnica brook in Samoborsko gorje Mts., Žumberak region, Central Croatia; UTM 33T WL 56; 8. May 1994.; leg. N, Matočec (holotype in ZA, isotype in NM); NM/4129 Motovunska šuma forest near Motovun, Istria region; UTM 33T VL 12; 20. Nov. 1998.; leg. N. Matočec.

## Discussion

Both collections of the species share several unique characters and could not be conspecific with any of known species, according to all important characters on specific level in genus *Scutellinia*, viz. ascospore shape and dimensions, ascospore ornamentation type and dimensions, marginal hair dimensions, marginal hair base construction and differentiation degree among marginal and excipular hairs (cf. Kullman, 1982 and Schumacher, 1990). Mentioned characters in both collections of *S. tuberculata* are presented in the following table.

| Coll. no. | Marginal hairs | | | Ascospores | | | Ascospore ornamentation | | |
|---|---|---|---|---|---|---|---|---|---|
| | base construction | length /µm | diam. /µm | shape * | shape ✝ | diam. /µm | shape | diam. /µm | height /µm |
| NM/ 2235 | bi- to tetra-furcate | 282-620 | 25.0-39.7 | glob. | glob. | 18.1-21.1 | tub. | 1.4-6.4 | 1.4-4.3 |
| NM/ 4129 | bi- to penta-furcate | 275-795 | 27.6-46.5 | glob. | glob. | 19.4-23.8 | tub. | 3.8-5.8 | 1.4-3.7 |

The apothecia from both collections are very small for the genus, e.g. 1,0-1,6 mm in diam. (NM/2235) and 1,4 mm in diam (NM/4129, single apothecium) and additionally, their hymenia are always pinkish to orange, never red. The hair differentiation is obvious in both collections, where marginal hairs are generally much longer than the excipular ones. However, the presence of hairs with multifurcate bases could easily be overlooked, due to their low density in each apothecium. It seems that this species could be distributed in warm temperate region, viz. submediterranean and continental mediterranoid areas (cf. Bertović & Lovrić 1992).

The species is not likely to be confused with other globose-spored *Scutellinia* species because of its unique combination of characters. The small and orange to pinkish apothecia, occurrence of multifurcate marginal hairs, medium sized perfectly globose ascospores, beset with very large hemisphere-shaped tubercles clearly distinguish the species. *S. trechispora* (Berk. & Broome) Lambotte has globose spores and multifurcate marginal hairs (500-2000 µm in length), but spores measure only 14-18 µm and bear unique bluntly conical to molariform ornamentation. The species is quite common in Croatia and Slovenia. *S. paludicola* (Boud.) Le Gal has the same spore ornamentation type with similar dimensions, but spores are much larger, 25-30 µm in diam., and the hairs, which have no differentiation between marginal and excipular ones are very short, 100-350 µm in length with simple or only exceptionally bifurcate base (Schumacher, 1990). This species is not recorded in Croatia so far. *S. barlae* (Boud.) Maire - a species that is also found in similar habitat in Croatia (Matočec, 1998) has nearly the same dimensions and the same shape of the ascospores. On the other hand, *S. barlae* has the same type and dimensions of the apothecial hairs as *S. paludicola* and it also differs from *S. tuberculata* in type and in dimensions of the spore ornamentation. *S. minor* (Velen.) Svrček and *S. tuberculata* have overlapping dimensions of the marginal hairs and the ascospores. Though, *S. minor* has unbranched to trifurcate hair-base and the ascospores are globose to subglobose, even in the living state (Matočec, 1998). Additionally, *S. minor* has different spore ornamentation type, e.g. roller-shaped to cylindric-obtuse, measuring 1.2-2.1 µm in diam. and 0.8-2.2 µm in height. Besides, the species has much larger apothecia (3-8 mm in diam.) and belongs to boreo-polar species group (Schumacher, 1990, 1993). It has only been recorded in subalpine zone in Croatia (Matočec et al., 1995).

On account of certain taxonomic characters, *S. tuberculata* can be placed in subg. *Scutellinia* (multifurcate, prominent marginal hairs structurally differentiated from excipular hairs), section *Globisporae* Svrček (ascospores globose, with prominent, isolated spore ornamentation) as a second member in the section, nearby *S. trechispora* (Berk. & Broome) Lambotte.

## Acknowledgements

I wish to thank the stuff of Croatian State Directorate for the Protection of Nature & Environment, which supported the fieldwork in Istria, where I have discovered the second collection of the species. The author is also indebted to Dr. Ain Raitviir (Tartu) for critically reading the manuscript, and to Dr. A. Ž. Lovrić for checking the latin diagnosis.

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MYCOTAXON

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# OPEN LETTER TO THE SCIENTIFIC COMMUNITY OF MYCOLOGISTS: PROVIDE VOUCHER SPECIMENS

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David L. Hawksworth[10], Egon Horak[11], Richard P. Korf[12],
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To help minimise invalid publication of newly proposed scientific names of fungi, KORF (1995) provided advice on how to guarantee valid publication, and offered a few simple guidelines for authors, reviewers, and editors. He regretted that "unfortunately many of the errors are committed by highly respected mycologists, and published in thoroughly respectable journals" and emphasised that "although the ultimate responsibility for publishing correct names lies with authors, clearly reviewers and editors are shirking their duties to advise authors of such errors prior to publication."

In order to be published validly, names must be introduced according to requirements of the International Code of Botanical Nomenclature (ICBN; GREUTER et al.

1994, 2000). Since 1990 it has been compulsory to deposit the vouchers for new species and infraspecific taxa, the name-bearing types, in a herbarium or other collection. It is generally accepted that such voucher specimens should be deposited in publicly accessible reference collections such as herbaria.

However, voucher collections are invariably necessary not only when new fungi are described, but also in connection with any scientific study, whether by taxonomists, systematists, physiologists, chemists, molecular biologists, pathologists, ecologists, clinicians, etc., dealing with organisms. It is essential to preserve voucher specimens as dried material or, where possible, in addition as permanently preserved living cultures. When none of the investigated material is preserved, it is impossible to confirm the identity of the investigated taxa. If species concepts have changed, it is particularly crucial to be able to re-identify the organism at a later time. There are several examples of entities once thought to be species but now revealed as species complexes, where the species concept has been or will be changed, including *Pisolithus tinctorius* (BURGESS et al. 1995) and *Paxillus involutus* (FRIES 1985, HAHN & AGERER 1999). In such cases, re-identification of the original material is indispensable in order to know which organism was studied so that previous work will continue to be relevant. In recent years molecular biological studies have a tremendous impact on systematics, taxonomy, and ecology. DNA sequences are frequently obtained from fungal cultures. Too often there is no record either of an exact citation of the fungal material used, such as an unequivocal number referring to collection accession data and the voucher culture, or reference to the institution where the material has been deposited. Often strain numbers are lacking in publications when sequences from GenBank are used. Frequently, only personal or laboratory strain numbers are given, which make it hard to trace the origin of the fungal material. Only accession numbers allocated by permanent public or other open institutional collections can ensure the retrieval of voucher material over the long-term. It is not yet common practice to publish complete collection or isolation data, or to deposit vouchers, except in taxonomic articles.

Conservation of dried fruit-bodies from which cultures are made is also indispensable in order to allow checking of anatomical and morphological features that cannot be reproduced in culture. The cultures also can be checked using molecular methods after prolonged preservation, in order to exclude the possibility of contamination. While it is rarely possible to culture fungi from dried specimens, the associated collection details are indispensable not only to clarify the geographical and ecological source, but also to facilitate the possibility of recollecting the fungus in the same site. This requires as detailed and exact a description of the sampling locality as possible, preferably including geographic co-ordinates, something now facilitated by hand-held or wrist-band global positioning devices.

Voucher specimens are equally important for a wide range of other investigations. DENNIS' (1960: xxii) remark that "records that cannot be verified are mere waste paper" applies to numerous aspects of our discipline. Studies of the species composition of any habitat depend on properly determined fungi, and so will require dried vouchers deposited in publically accessible collections. This applies, for example, not only to fruit-bodies, but indeed to any other form of fungal structure, such as sclerotia, or ectomycorrhizae (AGERER 1991) used in scientific work. Ecological, chemical, applied, and physiological studies quite often rely on ecotypes of species, which could later be considered, depending upon the species concepts applied, as separate species. In the seventies, HAWKSWORTH (1974), YOCUM & SIMONS (1977) and AMMIRATI (1979) were among the first to point out the importance of voucher material particularly in chemical, but also other physiological and ecological studies. In ecological studies on ectomycorrhizas, the increasing use made of RFLP patterns or DNA sequences for the detection of the symbionts requires comparison with those of identified fruit-bodies. In many studies, the identified ectomycorrhizae are completely consumed by the extraction and amplification methods. Instead, voucher specimens should be stored, when individual tips of a larger hyphal system have been used. Even more important is the citation and preservation of the fruit-body specimen from which DNA was extracted for comparison with that obtained from ectomycorrhizae.

Voucher cultures are urgently needed when clinically relevant fungi are investigated and their etiologic data and their impact on human beings have to be evaluated (DE HOOG & GUÉHO1985). Further, where cultural or chemical features are crucial for the evaluation of newly described fungi such as yeasts, the non-availability of cultures can make interpretation impossible and frustrate other researchers (BANNO et al. 1993, HAWKSWORTH 1984). Sufficient information on clinical direct microscopy or histopathology results to determine whether an isolate was medically significant or a biomedical contaminant is essential for later evaluations. In cases of apparently exotic fungi, a brief notation of relevant patient travel history is strongly recommended.

Additional documentation requirements apply to strains deposited in the major service collections of fungal cultures, such as ATCC (American Type Culture Collection, Manassas, Virginia, USA), CBS (Centraalbureau voor Schimmelcultures, Baarn/Utrecht, The Netherlands), or IMI (CABI Bioscience (UK Centre), Egham, Surrey, UK); these and other culture collections often provide forms for depositors to simplify the documentation process. In such major culture collections, the cultures are safely stored with cryo-preservation methods, and may be revived at any time. For sporulating fungi, the citation of the allocated accession number is generally enough to meet the goal of reproducibility of scientific results, *i.e.,* to confirm the identity of the species studied. But a comparison with naturally grown material is only possible when the original collection or isolation details have been cited. A completely different situation arises in cultures which are sterile and thus cannot be identified by normal methods. For such cultures, preservation of vouchers is particularly important together with exact collection data of the fruit-bodies and the herbarium or other collection where they have been deposited. Misidentifications can then be detected, new species concepts applied to the material, and recollection of new living material from the site of the original fruit-body might still be possible.

The addresses of public and open institutional dried reference collections and herbaria can be found in Index Herbariorum (HOLMGREN, HOLMGREN & BARNETT 1990), and of microbial culture collections in the World Directory (SUGAWARA et al. 1993); these works both contain generally applied acronyms, which are convenient and informative enough for citation. Public and institutional collections ensure that the material in their care is well-curated and preserved in a proper way for centuries, and they usually loan dried material free of charge, subject to certain requirements. Whilst the long-term maintenance of private herbaria is often uncertain and the mailing expenses exceed a private budget, nearly all of the international herbaria and other institutions that house fungi will warmly accept properly dried and documented fungal material. Living cultures are normally supplied for a charge to cover the cost of preparation and carriage, again subject to particular regulations that may apply; details vary and are available from the collections' catalogues and web sites.

Particularly in recent years, the behaviour of the scientific community has set tongues wagging, especially in relation to falsified data in publications concerning human cancer. It is a fundamental principle of science that research work must be reproducible. Reproducibility requires that studies can be made using the same dried material or cultures as the original study used. As a consequence, publications lacking unambiguous reference to the locations where the critical study material can be accessed by later researchers should not be accepted for publication. They are of no or limited scientific value in that they cannot be reproduced. Editors and referees in all aspects of mycology are often confronted with such situations and it is therefore necessary to include advice for the deposition of voucher material in instructions for authors (e. g. HAWKSWORTH 2000) and to regard this as a prerequisite for publication.

All scientists are responsible for their results. This responsibility lies not only in relation to the scientific community, but also in relation to those who support their research - the taxpayer, charities or other funding agencies, and ultimately society at large. The general public expects integrity from the scientific community. It is the responsibility of individual scientists, referees, and editors to rigorously apply the highest standards and make every effort to ensure that published research will be reproducible. Reproducibility in mycology is irrevocably and inextricably connected to the unequivocal citation of voucher specimens and cultures.

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# BOOK REVIEWS AND NOTICES[1]

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## GENERAL

**Funghi Ipogei d'Europa.** By Amer Montecchi & Mario Sarasini. May 2000. Associazione Micologica Bresadola, Via A. Volta 46, 38100 Trento, Italy. Pp. 714, numerous coloured plates. ISBN and price not indicated.

To those already familiar with Montecchi & Lazzari's *Atlante Fotografico di Funghi Ipogei* (1993), it will come as no surprise to learn that this is an extraordinarily well-illustrated and carefully executed piece of work. In this new book, while many of the colour photographs are reproduced from its predecessor, the coverage is extended both geographically and systematically. Further, the presentation has been totally revised with experience gained from that work, and all the text items are presented first in Italian and then immediately followed by an English translation. Keys at all levels are provided in the main body of the work, and quickly found as they are printed against a grey background; they are not gathered at the back as before, and the German translation of the keys has been dropped. For each species, the scientific name with author and date (a zoological practice being promoted in *Mycological Research* as it provides a short-cut to the precise bibliographic reference) is followed by its etymology, a detailed description, and information on the habitat, herbaria in which material has been seen, taxonomic notes, and remarks. The fine colour photographs include ones of the habit, generally with one fruit body sliced into two, and also the spores (in some cases with SEM micrographs).

A very broad view of hypogeous fungi is adopted, and while I was at first surprised to find, for example, *Peziza pseudoammophila* and *Geastrum triplex,* on reflection this can only benefit the field mycologist who finds such fungi bursting through the soil surface. As well as ascomycete and basidiomycetes, the endomycorrhizal-forming *Endogonales* are also covered here. In all, 179 species are treated, 66 more than in the 1993 book.

An extensive bibliography is provided, and the dates of names given appear to have been carefully checked. Regrettably the authors fall into the common trap of repeating author citations after the species name element in names of infraspecific taxa other than those that include the type of the species itself and repeat the specific name unchanged. They also to some extent misunderstand the type method which they imply should represent the range of developmental stages or other variants. They are scathing of researchers finding that a type does not accord with the characters mentioned in the literature and so wishing to change names. Conscious of the importance of careful determinations, the authors cite specimens they have seen, and have preserved ones they have collected and studied. However, they do not cite references to reported distributions or even the occurrences of the species in the different countries of Europe. It would be helpful to mention such reports, even cautiously, in any future revision.

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[1] Books for consideration for inclusion in this column should be sent to the Book Review Editor at his permanent address and **not** to Madrid: David L. Hawksworth, MycoNova, 14 Finchley Lane, Hendon, London NW4 1 DG, UK; tel/fax [+44] (0) 20 8203 4282; e-mail myconova@btinternet.com. Unsigned reviews and notices are by the Book Review Editor.

Overall, this book is a most remarkable achievement for two 'amateur' mycologists who happen to share an extraordinary enthusiasm for truffles and truffle-like fungi. It is destined to be accepted as the standard reference work for the identification of these fungi in Europe for decades to come. If you look for hypogeous fungi in the field, or indeed just enjoy seeing top-quality colour photographs of unusual fungi, this is a must for your bookshelves.

**International Code of Botanical Nomenclature (Saint Louis Code) adopted by the Sixteenth International Botanical Congress, St Louis, Missouri, July-August 1999.** Edited by W. Greuter, J. McNeill, F. R. Barrie, H. M. Burdet, V. Demoulin, T. S. Filgueiras, D. H. Nicolson, P. C. Silva, J. E. Skog, P. Trehane, N. J. Turland & D. L. Hawksworth. June 2000. [Regnum Vegetabile Vol. 138.] Koeltz Scientific Books, D-61453 Königstein, Germany. Pp. xviii, 1-474. ISBN 3 904144 22 7, ISSN 0080-0694. Price DM 80.

This is the single book every reader of *Mycotaxon* needs to own or have ready access to. The *Code* is revised after long and often intense debates by the Nomenclature Section that precedes every six-yearly International Botanical Congress; the previous one was held in Yokohama, Tokyo, in 1993. It is critical always to have the current *Code* on hand as rules and examples change, often in subtle ways. Having said that, the hallmark of the St Louis Section meeting in 1999 was one of extreme conservatism; not only were proposals for protected lists of names in current use rejected, but also the provisions for the registration of newly published names passed in Tokyo were deleted. Edited versions of all the discussions are being published separately (*Englera* **20**: in press, 2000) for those wishing to know the background to the changes made or sense the passion and even acrimony in some debates.

The most important changes affecting mycologists in the St Louis *Code* relate to a reworking of the section on the typification of species and infraspecific names, including new definitions of what constitutes a type or gathering, for example when there are several pieces in a single preparation. Provisions relating to cases where a culture permanently preserved in a metabolically inactive state is designated as a type have been taken into the body of the *Code*, the unnecessary introduction of anamorph names is counseled against, and the use of author citations of names in non-taxonomic works is discouraged. As in recent editions, scientific names in all ranks are printed in *italic* type, a practice already established in some botanical and mycological journals and which makes it possible to distinguish at a glance, for instance, *Fungi* (= the kingdom) from fungi (=organisms studied by mycologists).

The cover is black for reasons that will become apparent to all who read the Preface. As Secretary to the *Code*'s Editorial Committee, it would not be appropriate for me to comment further on this aspect here.

## ZYGOMYCETES

**Illustrated Genera of *Trichomycetes*. Fungal symbionts of insects and other arthropods.** By J. K. Misra & Robert W.Lichtwardt. Science Publishers Inc., P. O. Box 699, Enfield, NH 03748, USA. Pp. x + 155. Spiral bound. ISBN 1 57808 080 0. Price US $ 29.95, £ 19.95.

The trichomycetes, used by the authors in lower case to indicate that these organisms are not a natural group, are amongst the least studied fungi. Yet their fascinating life-style in the hind guts of a wide variety of arthropods in terrestrial, marine and freshwater habitats, combined with the beauty of their spores, they might have been expected to have attracted more attention. A limiting factor has been the availability of identification aids. This clearly presented book, made possible by a US PEET grant to promote taxonomy, aims to provide an entrée into this fascinating world not only for mycologists but also for entomologists, invertebrate pathologists and symbiologists.

Descriptions of orders and families precede keys to the 55 genera. For each genus there is one page with a short description, the name of the type species, information on hosts and distribution, a list of known species, and literature references. Places of publication of ordinal and family names are provided, and those of the type species of each genus, but not the genus itself (although often in practice the same place as that for the type species); those wishing to use author citations from this work should, however, note that the names of authors have not been abbreviated according to the internationally recommended standard. The facing page is occupied by first-rate phase contrast photographs of the type or another species of the genus. The hosts are also tabulated, ranging from fly larvae to pill bugs, crayfish, fiddler crabs, and millipedes. A summary of collection and culture methods is provided, together with an extensive bibliography.

Based on Lichtwardt's unparalleled knowledge of these enigmatic fungi, the work is clearly authoritative and provides a sound and up-to-date basis for the exploration of these fungi.

## ASCOMYCETES

**A Monograph of the Genus *Pezicula* and its Anamorphs.** By Gerard J. M. Verkley. December 1999. Centraalbureau voor Schimmelcultures, P. O. Box 273, NL-3740 AG, Baarn, The Netherlands. [Studies in Mycology No. 44.] Pp. 180, 50 figs (2 in colour). ISBN 90 70351 40 4, ISSN 0166-0616. Price Hfl 85.

Monographs are the backbone of systematic mycology and if they are to stand the test of time then they need to consider as many possible approaches to the group as possible to provide a robust treatment. This revision is a model in that respect as it combines traditional approaches with a molecular study, data from the anamorphs, studies on single-ascospore isolates, and cultural characteristics.

Twenty six species are accepted in *Pezicula*, of which two species and one subspecies are newly described, four in *Neofabraea*, and one in the new genus *Scleropezicula* which is introduced to the single species *S. alnicola*. . *Neofabraea* and *Pezicula* differ in having, respectively, poorly *vs.* well-delimited apothecial disks, narrowly cylindrical *vs.* cylindrical clavate to clavate asci, and *Phlyctema vs. Cryptosporiopsis* anamorphs. Both *Neofabraea* and *Pezicula* have a basal stroma with thin-walled cells in which the lumina are never less than two thirds of the cell diameter, whereas in *Scleropezicula* the basal stroma consists of thick-walled cells in which the lumina are less than one third of the cell diameter. *Scleropezicula* has a distinctive anamorph with sympodially proliferating conidiogenous cells for which the new generic name *Cryptosympodula* is introduced.

Twenty of the accepted *Pezicula* species have known *Cryptosporiopsis* anamorphs, 12 of which have separate scientific names. I was pleased to see that Verkeley refrained from introducing binomials for those not already named. He did, however, accept seven additional already named species of *Cryptosporiopsis* for which no *Pezicula* teleomorphs are currently known.

The molecular study examined 90 strains of 21 taxa which were characterized by RFLP patterns of the small subunit and ITS1/4 region of the nuclear ribosomal RNA gene cluster. The resultant dendrograms fully supported the three-genus taxonomy adopted based on differences in cellular structures, ascus shape, and the anamorphs.

Separate keys to teleomorphs, anamorphs, and those known in culture are included. The keys are strictly dichotomous and the separations in general seem clear although I have not yet had a chance to test them with fresh collections from the field

Bibliographic studies and investigations of typifications have been meticulously carried out. Inevitably there are changes in names, not relating to new information on relationships but consequent on applications of the rules in the *Code* relating to priority. Such changes can be unfortunate where the species involved are familiar, but since 1993 provisions have existed in the *Code* to enable more names to be safeguarded. However, it is right for authors to decide whether it is best to allow a change or not. For example, in this work, the well-known name *Pezicula livida* is shown to be a synonym of *P. cinnamomea*. However, it was prudent to make this change rather than seek protection as Verkley found that *P. livida* had been applied to two species, here called *P. cinnamomea* (asci 8-spored) and *P. eucrita* (asci 4-spored). Had he opted to seek retention of *P. livida,* in the future there would always be some uncertainity of whether that name was being applied in the old broad or restricted new sense; with the treatment he adopts there will be a clean break with the past.

There is a necessary book-keeping section on Excluded or Insufficiently Known Taxa with names published in *Dermea, Ocellaria,* or *Pezicula* which are not treated as synonyms of any of the accepted species. Six such names are considered distinct species in other genera and new combinations made into *Calycina* (for *P. dennisii* on *Urtica*; clearly wrongly described by the young Hawksworth in *Pezicula*), *Mycosphaerangium* (three species), *Ploettnera,* and *Sageria.*

The line drawings and photomicrographs are prepared to the highest standards and give the presentation a most pleasing appearance. My one regret is that amongst the fine illustrations there was only one photograph showing the habit of an apothecium, and that was a half-tone. A page of coloured macro-lens photographs of the distinctive colours of the mature apothecia of as many species as fresh material was available would have complemented those showing the colour of cultures. This would have made the work more appealing to the many amateur discomycetologists that will need to use this work to identify their collections.

This is the first major work Verkley has produced since his 1995 PhD on the ascus apex in three families of *Leotiales*, which included extensive information on ultrastructural features. It is gratifying that he has been able to move into monographic work so successfully in such a short time. He has set very high standards here and we will keenly await his future treatments of other genera.

**Genera of Ascomycetes from Palms.** By Kevin D. Hyde, Joanne E. Taylor & Jane Fröhlich. July 2000. Fungal Diversity Press, Centre for Research in Fungal Diversity, Department of Ecology and Biodiversity, University of Hong Kong, Pokfulham Road, Hong Kong. Pp. vii + 247. ISBN 962 85677 3 3X. Price US $ 100.

The identification of small tropical ascomycetes, especially those with small perithecia, can be particularly frustrating for the non-specialist. Where can one go to quickly gain an idea of what the candidate genera for a particular specimen might be? This is an especial problem for palms and bamboos which are so rich in perithecial ascomycetes. Now there is a substantial contribution to assist those facing this situation with palms. Three mycologists with deep interests in palm fungi have combined forces to introduce 100 selected genera of ascomycetes which occur on palms. Two pages are devoted to each genus: a description, type species, any anamorphs, distribution, notes, and literature references on the left, photographs of the type or another species on the right. In addition there is a section on microscopic examination, a key to the genera covered, and an illustrated glossary. While the literature cited will have to be used to make confident species determinations, and all ascomycete genera known on palms are not covered, there is no question that this work will be a major short-cut to identifications for mycologists of all kinds working with palms, including plant pathologists concerned with disease problems in coconut, date palm, or oil palm. Further, many of the genera covered also occur outside the tropics and on different host plants. The work will therefore also be found of value in fungal diagnostic laboratories generally. Well-presented and hardbound, my main concern over the volume is the price of what is essentially an introductory work rather than an exhaustive monographic treatment. At US $ 100 each, I wonder how many copies will actually find their way into the tropical laboratories that would most benefit from having it on their shelves; I fear it may be too few.

## LICHEN-FORMING FUNGI

**Lichens of Rainforest in Tasmania and South-eastern Australia.** By G. Kantvilas & S.J. Jarman. 1999. Australian Biological Resources Study, G. P. O. 787, Canberra, ACT 2601, Australia. [Flora of Australia Supplementary Series No.9.] Pp.212, coloured plates. ISBN 0 642 56802, ISSN 1323 2169. Price not indicated.

The core of this book is an annotated series of 217 coloured photographs (some with magnified insets) taken by B. A. Fuhrer, mainly of macrolichens, that occur in cool temperate rainforests in the region. The genera are arranged alphabetically, and following a short introductory paragraph on each, notes on the diagnostic features and habitats of the selected species are provided. The style is very user-friendly, employing minimal technical terms, and has spaciously laid-out pages which should encourage rainforest ecologists and naturalists to take more note of lichens. A dichotomous key covering the macrolichens of Tasmanian rainforests is included near the back of the book; this includes some species not covered in the main text. I was especially pleased to see the first sections of the book which are again well-illustrated by excellent colour photographs and cover not only lichen structure and biology, but further lichen habitats, communities, nutrition, contribution to chemical cycles (particularly atmospheric nitrogen fixation), and conservation. A glossary and list of further reading precede the index. A characteristically detailed Foreword by D. J. Galloway covers historical aspects of the investigation of Tasmanian lichens.

**The Lichens and Lichenicolous Fungi of Belgium and Luxembourg: an annotated checklist.** By Paul Diederich & Emanuel Sérusiaux. 2000. Museé National d'Histoire Naturelle, 25 rue Munster, L-2160 Luxembourg, Luxembourg, Pp. 207. ISBN 2 919877 00 3. Price not indicated.

This is primarily a checklist covering 1151 accepted taxa, of which 930 are lichens (21 of those lichenicolous lichens which live on other lichens) and 201 lichenicolous fungi. For each species information is given on synonyms, habitats (with colour photographs), distribution, and literature references. However, it is much more than that. The first 63 pages include an historical overview of lichenological exploration in the two countries, details of the area covered, and analyses of the data. While the main body of the checklist is in English, the introductory sections are in three languages - English, Flemmish and French. Fifty accepted species have been described as new to science from the two countries, of which 32 are lichenicolous - mainly a tribute to Diederich's keen eye. While this shows how much novelty is new is still to be found even in extensively disturbed and relatively well-studied areas of Europe, it is sad to note that 49 lichen species are regarded as extinct.

**Katalog lišejníků České republiky. A Catalogue of Lichens of the Czech Republic.** By Antonin Vězda & Jiří Liška. 1999. Institute of Botany, Academy of Sciences of the Czech Republic, CZ-252 43 Průhonice 1, Czech Republic. Pp. 283. ISBN 80 86188 03 5. Price not indicated.

An extraordinarily detailed catalogue prepared in Czech by two of Europe's most respected lichenologists. 1534 species in 255 genera are accepted. For each synonyms used in the country's literature are listed, and all citations to the literature supporting those records are provided - a staggering 816 references. No information on habitat or distribution is provided, but could be located from the cited literature. The taxonomic treatment is conservative, with segregate genera such as *Cladina* and *Melanelia* not being adopted. Lichenicolous lichens are included, but not lichenicolous fungi. Because of the extensive citations of synonyms, the work is sure to be used as a short-cut in many parts of central Europe by those wishing to know what unfamiliar nineteenth century names actually refer to.

## BASIDIOMYCETES

**Die *Amanita*-Arten von Südwestchina.** By Zhu-Liang Yang. 1997. J. Cramer in der Gebrüder Borntraeger Verlagsbuchhandlung, D-14129 Berlin, Germany. [Bibliotheca Mycologica Vol. 170.] Pp. 240, 175 figs. ISBN 3 443 59072 X. Price not indicated.

A detailed treatment well-illustrated by line drawings recognizing 47 species, of which nine are formally described as new to science, and four are allocated provisional names but not given Latin diagnoses; this last practice is one I find particularly unfortunate as the names inevitably enter the scientific literature whether they meet the criteria for the valid publication of names or not. Fifteen of the taxa are new to China, an indication of how little we know of the mycobiota of this area. Indeed, only ten species of the genus were accepted for the entire country in S. C. Teng's *Fungi of China* (1996). This is, however, much more than a regional monograph as a modified infrageneric classification is adopted. The genus is divided into subgenera *Amanita* (with sections *Amanita, Caesareae* and *Vaginatae*) and *Lepidella* (with sections *Amidella, Lepidella, Phalloideae* and *Validae*). Section *Mappae* was included in section *Validae*. Full descriptions are provided, and while there are no photographs or coloured drawings, the line drawings are particularly carefully executed and include details of the hyphal systems. While publication in this column is a way of ensuring the work is widely known outside China, I worry about the accessibility of such texts within China on the grounds of both cost and language. Although published three years ago, this work is noted here at the specific request of the author who wished his work to be more widely known.

## MISCELLANEOUS

**Ecology of Fungi.** Edited by D. J. Bhat & S. Raghukumar. 2000. Goa University, Goa, India. Pp. iv + 192. ISBN and Price not indicated.

This work represents the proceedings of a symposium on Fungal Ecology held at Goa University in January 1998 on the occasion of a meeting of the International Mycological Association's Committee for Asia and the Mycological Society of India. It contains 28 papers, including a plenary lecture in which John Webster reflects on the advances made in fungal ecology over the last 50 years. Although primarily of interest to the fungal ecologist, the book does include several short papers on aspects of fungal diversity and records of interest. Books such as this, which are not produced through a publishing house operating internationally or have been allocated an ISBN number, rarely come to the attention of the wider mycological audience. For this reason I felt it appropriate to draw attention to this work in this column.

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